The Evolution of Biomarkers to Guide the Treatment of Metastatic Colorectal Cancer

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Introduction

Colorectal cancer (CRC) is the third most common type of newly diagnosed cancer and the third most common cause of cancer-related death in the United States, with 50,630 deaths due to CRC expected in 2018.[1] Overall CRC incidence and death rates have been declining over the past several decades.[2] The decreasing trend in CRC mortality rate is attributed to increased screening and use of colonoscopy in screening, improvements in treatment, and greater integration of continuity of care.[3] Changes in patterns of lifestyle habits associated with CRC risk and increased usage of screening have contributed to a decline in the overall incidence of CRC by about 3.5% annually over a 10-year period from 2005 to 2014. However, the incidence of CRC among patients younger than 55 years has been increasing since the mid-1990s, with the most rapid increase in metastatic disease.[4] This steady increase in CRC incidence among younger adults, which may be due to obesity and lifestyle factors, is a concern for the future burden of disease and colorectal cancer mortality in this population.[4] Approximately 50% to 60% of patients with CRC develop metastatic CRC (mCRC), and 80% to 90% will have unresectable liver metastases.[5,6]

Although currently available therapies can reduce rates of disease progression and extend survival of patients with mCRC, the possibility of a cure for mCRC is limited to select individuals who are able to undergo surgical resection of metastatic disease.[5] The 5-year relative survival for patients with mCRC is about 13.9%.[2] Alterations in several key CRC genes are linked to prognosis and survival, but importantly also serve as biomarkers to identify tumor sensitivity and resistance patterns to targeted therapies. These statistics highlight the critical need for better therapies, as well as for biomarkers to guide treatment decisions that improve treatment outcomes for mCRC. Recent advances in biomarker research and new treatment options for this disease warrant the education of healthcare personnel treating patients with mCRC so that they can better tailor therapies for more effective management.

Genetic Biomarkers in CRC

The development of CRC is driven by molecular changes and mutations in key genes within a network of signaling pathways that

ABSTRACT

In the United States, colon cancer is one of the leading causes of death and cancer-related death. There is a critical need to improve clinical outcomes in patients with metastatic colorectal cancer (mCRC), as current survival rates are unsatisfactory. There have been significant advances in the treatment of mCRC over the past decade. Molecular characteristics of mCRC and identification of mutations can serve predictive and prognostic indicators of disease response to treatment. These biomarkers can be incorporated into clinical decision making when developing an individualized treatment plan. Targeted therapies have improved the survival of patients with mCRC. As we learn about the various molecular alterations in this disease, additional emerging therapies can be developed to improve clinical outcomes in patients with mCRC.

TABLE 1. NCCN-Recommended Tumor Biomarker Testing and Impact on Treatment[6]

Biomarker/ Gene	Impact on Treatment Selection
KRAS	Mutant *KRAS* tumors should not be treated with cetuximab or panitumumab
NRAS	Mutant *NRAS* tumors should not be treated with cetuximab or panitumumab
BRAF V600E	*BRAF* V600E tumors are unlikely to respond to cetuximab or panitumumab
MMR or MSI testing	Patients with stage II MSI-H tumors have a good prognosis and do not appear to benefit from 5-FU adjuvant therapy. Tumors with deficient MMR or MSI-H status may respond to anti-PD-1/PD-L1 immunotherapy

5-FU indicates fluorouracil; *BRAF*, V-raf murine sarcoma viral oncogene homolog B1; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; MMR, mismatch repair; MSI, microsatellite instability; MSI-H, microsatellite instability-high; NCCN, National Comprehensive Cancer Network; *NRAS*, neuroblastoma RAS viral (v-ras) oncogene homolog; PD-1/PD-L1, programmed cell death-1/programmed cell death ligand-1.

influence tumorigenesis.[7] Our understanding of the biology of CRC continues to improve, and efforts to develop therapeutic strategies that target some of these genetic mutations have led to advances in treatment. Genetic testing to select therapies for patients with CRC has been the focus of many recent studies and has become standard practice for the management of patients with CRC.[8] With the availability of effective immunotherapy for mCRC and newer targeted therapies, there has been increased acceptance of the need for more extensive molecular testing. Given the wide array of genetic and epigenetic alterations involved in colorectal tumorigenesis, efforts to classify CRC based on distinct subtypes that represent pathologic and molecular features have been challenging.[7] Most recently, however, a molecular classification for CRC that defines 4 different subtypes with implications for patient management has been reported.[9] Several biomarker tests provide prognostic and/or predictive information for patients with CRC (**Table 1**[6]).

Biomarkers that can predict the response to specific therapy or treatment regimens are predictive. Kirsten rat sarcoma viral oncogene homolog (*KRAS*) and neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*) gene status in the epidermal growth factor receptor (EGFR) signaling pathway are predictive for tumor sensitivity to monoclonal antibodies that target the EGFR. Wild-type *KRAS* and *NRAS* tumors respond to these therapies, while mutant *KRAS* and mutant *NRAS* tumors do not respond.[8] DNA mismatch repair (MMR) gene status is predictive for tumor sensitivity to anti-programmed cell death-1/programmed cell death ligand-1 (PD-1/PD-L1) therapies.[7] Mutations or epigenetic modifications of MMR genes can result in MMR protein deficiency and microsatellite instability (MSI). Depending on the extent of MSI, tumors are classified as MSI-high (MSI-H) or MSI-low (MSI-L). Tumors

that are MMR protein deficient are considered MSI-H. Sufficient MMR proteins are critical for deleting DNA mismatches that occur during DNA replication; therefore, tumor cells that are MMR protein deficient accumulate thousands of mutations that can encode for mutant proteins that promote their growth. These proteins have the potential to be recognized and targeted by the immune system.

Mutations in other genes, including V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*), phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (*PIK3CA*), phosphatase and tensin homolog (*PTEN*), overexpression of V-Erb-B2 erythroblastic leukemia viral oncogene homolog 2 (*HER2*), and mesenchymal-epithelial transition (*MET*), may also influence response to anti-EGFR monoclonal antibodies, but only *BRAF* testing is included in current guidelines.[8]

Certain molecular alterations in the tumors have significant implications for patient treatment and "personalized therapy" for CRC. However, the use of tumor-based gene mutations to guide therapy can be influenced by the source of tumor DNA, sampling sites, and temporal factors. Although the most common source of DNA for molecular profiling is isolated from formalin-fixed paraffin embedded (FFPE) tumor tissue specimens, cell-free DNA, which is believed to reflect circulating tumor DNA (ctDNA), can be isolated from whole blood, that is, "liquid biopsy."

During the progression of mCRC, different patterns of genetic mutations may develop between DNA collected from primary tumors and sites of metastatic disease.[7] For mutations in certain clinically relevant genes such as *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, and mutant p53 (*TP53*), there is a greater than 90% concordance between primary tumors and metastatic disease.[7,10] Concordance for PTEN expression by immunohistochemistry (IHC) is lower. Rates of PTEN expression vary from 47% to 98% between primary and metastatic sites, thereby reducing the utility of this biomarker in clinical practice.[7,11]

The American Society for Clinical Pathology, the College of American Pathologists, the Association for Molecular Pathology, and the American Society of Clinical Oncology have developed evidence-based guidelines to establish standard molecular biomarker testing to guide targeted therapies for patients with CRC.[8] According to their guidelines, the evidence supports molecular testing for genes in the EGFR signaling pathway such as *KRAS* and *NRAS*. They noted that mutations in other biomarkers also have clear prognostic value. These markers include testing for the *BRAF* pV600, DNA MMR gene status and IHC testing for MutL homolog 1 (MLG1), MSH6 and PMS2.[8] The National Comprehensive Cancer Network (NCCN) guidelines specifically recommend universal MMR or MSI testing in all patients with a personal history of colon or rectal cancer.[6] The NCCN also recommends that patients with mCRC have their tumors genotyped for *RAS* (*KRAS* and *NRAS*) as well as the *BRAF* V600E mutation.[6] The development of validated, robust biomarkers is essential for personalized therapy.

TABLE 2. CRC Molecular Subtypes[7,9,12,13]

Subtype	Biologic Taxonomy	Percentage of Patients	Tumor Characteristics	Anatomic Location	Prognosis	Treatment Implications
CMS1	MSI immune	14%	Hypermutated; MSI high; strong immune activation; *BRAF* mutation	Right-sided	Good prognosis, but poor survival after relapse	Likely benefit from immune checkpoint blockade
CMS2	Canonical	37%	Epithelial; high chromosome instability with microsatellite stability; marked WNT/MYC pathway activation, *TP53* mutation and *EGFR* overexpression	Left-colon and rectum	Good survival after relapse	Anti-EGFR therapies likely effective; may respond to oxaliplatin-based regimens
CMS3	Metabolic	13%	Epithelial; metabolically dysregulated; *KRAS*, *PIK3CA*, and *IGFBP2* mutations	No predominance	Intermediate survival	Likely resistant to anti-EGFR therapies
CMS4	Mesenchymal	23%	MSI heterogeneous; prominent TGF-β activation, angiogenesis, and stromal infiltration; NOTCH3/VEGFR2 overexpression	Typically diagnosed at more advanced stages	Worst overall and relapse-free survival	May be responsive to antiangiogenic therapies and perhaps more sensitive to irinotecan-based regimens

BRAF indicates V-raf murine sarcoma viral oncogene homolog B1; CMS, consensus molecular subtype; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; MSI, microsatellite instability; NOTCH3, neurogenic locus notch homolog protein 3; VEGFR, vascular endothelial growth factor receptor.

mCRC Molecular Subtypes

Gene expression-based subtyping is an accepted process for stratifying diseases; however, published gene-expression classifications showed a high level of heterogeneity and lack of consistency among subtypes of CRC. In 2015, the Colorectal Cancer Subtyping Consortium (CRCSC), an international consortium, was formed to resolve inconsistencies among the reported gene expression-based subtype classifications of CRC.[9] They found interconnectivity among 6 different classification systems. Using this interconnectivity, they developed 4 consensus molecular subtypes (CMS) of CRC with distinguishing features (**Table 2**[7,9,12,13]). Their aim was to establish a useful cancer subtyping strategy that incorporates clinical and molecular features that correlate with patient outcomes.

Implications for Therapy

These 4 consensus molecular subtypes (CMS1-4) are biologically distinct, have different clinical courses, and may predict therapy response.[13] Patients with CMS1 subtype have MSI-H tumors that produce mutated proteins due to the high number of gene mutations. These mutated proteins are recognized by the immune system and result in a lymphocytic infiltrate of the tumor.[6,12,14] Therefore, patients with CMS1 tumors may be excellent candidates for immune checkpoint inhibitors.[12,15] A recent phase 2 clinical trial of pembrolizumab (PD-1 inhibitor) demonstrated a 40% response rate in patients with MMR-deficient mCRC. There was no response in patients with MMR-proficient tumors.[12,16] Patients with tumors that are MMR protein deficient or MSI-H mCRC usually have a poor prognosis and are less responsive to conventional chemotherapy.[17,18]

Patients with CMS2 subtype disease do not typically harbor tumor mutations of *BRAF* or *RAS*, and are therefore likely to benefit from anti-EGFR therapies. Patients with CMS2 tumors also may benefit from oxaliplatin-containing regimens, although this observation was conducted from a retrospective analysis of patients with early stage colon cancer and requires further confirmation.[19] Given frequent activation of c-MYC proto-oncogene (MYC) and WNT signaling pathways, CMS2 tumors may respond better to a number of agents under development that target these pathways, as well as inhibitors of Aurora A kinase.[12,20]

Patients with CMS3 subtype tumors have frequent *RAS*, *PIK3CA*, and *PTEN* mutations, which confer resistance to anti-EGFR therapy. However, these tumors show an increased activity in various metabolic pathways that are under investigation as new targets for agents that target tumor glycolysis, glycogen synthase kinase, and amino acid metabolic pathways.[12,21-23]

CMS4 tumors express genes associated with epithelial to mesenchymal transition (EMT), which appears to be higher in stromal cells as compared with tumor cells. Agents that inhibit the TGF-β signaling pathway may be useful in affecting tumor stroma. CMS4 tumors may also be sensitive to inhibitors of angiogenesis. Because they are also enriched with stem cells, CMS4 tumors appear to be particularly sensitive to combinations of irinotecan, fluorouracil, and leucovorin, such as FOLFIRI and FOLFOXIRI.[12,24]

Systemic treatment for CRC involves various cytotoxic drugs and monoclonal antibodies (mAbs), administered as single agents or in combination. At diagnosis of mCRC, tumor biomarkers should be tested (Table 1), as the choice of therapy is influenced by the

TABLE 3. NCCN Recommendations for Adjuvant Chemotherapy for Resectable Colon Cancer[6]

Stage of Disease	Treatment Options
Stage I	Adjuvant systemic therapy not recommended
Stage II, low-risk, MSI-H disease	• Adjuvant systemic therapy not required • Participate in clinical trial • Consider capecitabine or fluorouracil (5-FU)/leucovorin (LV)
Stage II, high-risk disease	Consider: • Capecitabine • 5-FU/LV • 5-FU/LV/oxaliplatin (FOLFOX) • Capecitabine/oxaliplatin (CAPEOX) • Bolus 5-FU/LV/oxaliplatin (FLOX)
Stage III	• 5-FU/LV/oxaliplatin (FOLFOX) • CAPEOX • FLOX • Capecitabine • 5-FU/LV

MSI-H indicates microsatellite instability-high; NCCN, National Comprehensive Cancer Network.

mutational profile of the tumor.[6] However, in clinical practice, the utility of these biomarkers is limited to identifying patients who should not receive anti-EGFR mAbs and patients for whom anti-PD-1/PD-L1 immunotherapy is appropriate. As more treatment options have become available, therapy response prediction and optimal patient selection is increasingly important to avoid unnecessary toxicities and healthcare costs.[13] The clinical utility of using these 4 consensus molecular subtypes (CMS1-4) to predict therapy response requires validation in prospective, independent clinical trials. Although the NCCN does not recommend the use of CMS molecular subtype classifications in clinical practice, it warrants further study as a platform to help guide appropriate treatment.

Treatment Options for CRC

Both NCCN and the European Society for Medical Oncology (ESMO) have released updated guidelines for the treatment of mCRC.[3,6] The treatment of mCRC is driven by disease presentation, features of the tumor, patient characteristics and expectations, and treatment preferences.[3,6] No single treatment regimen is preferred over another for initial treatment of metastatic disease.[6] Also, there are no data to suggest that clinical outcomes are different for patients who receive initial treatment with intensive therapy as compared with less intensive initial therapy followed by subsequent intensive therapies. This contrasts with the preferred initial approach to treatment for CRC in general, in which the use and types of systemic therapy are more limited. Also, the initial management of CRC is further defined by the primary tumor site. Treatment for patients with cancers that arise in the rectum (ie, rectal cancer) differs from

treatment of cancers that arise in the colon (ie, colon cancer) in that treatment for nonmetastatic, resectable rectal cancer includes the use of radiotherapy.[25] Radiotherapy is given to further decrease the risk of local tumor recurrence, which is higher for rectal cancers as compared with colon cancers. Radiotherapy may be administered pre- or postoperatively and either alone, sequentially, or concurrently with chemotherapy. The chemotherapeutic agents used are the same as those used for colon cancer.

Patients with nonmetastatic, resectable CRC who undergo complete surgical resection may be candidates for systemic postoperative adjuvant therapy, depending on the stage of their disease (**Table 3**[6]). Adjuvant chemotherapy should be administered for 6 months following the surgery. If chemotherapy is given preoperatively, such as for rectal cancer, perioperative treatment should total 6 months. Recommended systemic therapies include only fluoropyrimidines (eg, capecitabine, fluorouracil) and oxaliplatin (discussed later in greater detail relative to mCRC). The use of other cytotoxic drugs or mAbs in the adjuvant setting outside of a clinical trial is not recommended.[6] Biomarker testing for MSI-H tumors for stage II colon cancer is recommended, and can be useful to identify patients for whom adjuvant systemic therapy is not required.

Systemic therapy for advanced and mCRC involves the use of chemotherapy, targeted therapies, and immunotherapies. Surgery is not recommended for mCRC, but may be performed in selected cases for tumor-related symptoms or curative intent. Systemic therapies include multiple active drugs, either in combination or as single agents: fluorouracil (5-FU)/leucovorin, capecitabine, irinotecan, oxaliplatin, bevacizumab, cetuximab, panitumumab, ziv-aflibercept, ramucirumab, regorafenib, trifluridine-tipiracil, pembrolizumab, and nivolumab.[6] Broadly, these agents can be categorized as cytotoxic chemotherapy (5-FU, capecitabine, irinotecan, oxaliplatin, and trifluridine-tipiracil), targeted therapy (anti-EGFR: cetuximab, panitumumab; anti-VEGF/VEGFR: bevacizumab, ziv-aflibercept, ramucirumab, regorafenib), and immunotherapy (pembrolizumab, nivolumab).

Chemotherapy

Fluoropyrimidines, capecitabine, and 5-FU comprise the backbone of most chemotherapy regimens used for mCRC. These agents undergo metabolic conversion to inhibit thymidylate synthase, the rate-limiting enzyme for pyrimidine nucleotide synthesis, ultimately inhibiting DNA synthesis and repair.[26] Leucovorin is given with 5-FU to potentiate thymidylate synthase inhibition, thus potentiating the cytotoxic effects of 5-FU. Capecitabine is an oral prodrug of 5-FU that, when given twice daily, mimics the pharmacologic effects of continuous infusion 5-FU. The spectrum of adverse events (AEs) common to both agents, primarily diarrhea, mucositis, myelosuppression (neutropenia), and hand-foot syndrome, can be modulated with the schedule of 5-FU infusion.

Irinotecan is converted to an active metabolite, SN38, which binds to topoisomerase I, an enzyme that is critical for DNA replication and transcription.[27] Topoisomerase I inhibition causes DNA strand breaks, ultimately leading to DNA fragmentation and tumor cell death. SN38 is inactivated via glucuronidation by UDP-glucuronosyltransferase 1A1 (UGT1A1) and is then eliminated. A polymorphism in UGT1A1 (UGT1A1*28) is associated with reduced expression of UGT1A1 and hence there is increased toxicity due to decreased SN38 glucuronide. Predominant AEs include myelosuppression (neutropenia) and diarrhea. Oxaliplatin is a platinum analog that acts as an alkylating agent forming cross-links between DNA strands, leading to DNA damage and tumor cell death.[26] AEs include myelosuppression (neutropenia), cumulative peripheral neuropathy, and an acute, cold-related neuropathy. When used for CRC, oxaliplatin is always administered in combination with a fluoropyrimidine. Trifluridine-tipiracil is a newer fluoropyrimidine that will be discussed in greater detail.

Targeted Therapy

Molecular targets in CRC include the EGFR and the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR). Cetuximab and panitumumab are mAbs that target the EGFR, which is overexpressed in the majority of CRC.[6] Cetuximab is a chimeric mAb, whereas panitumumab is fully humanized. The epidermal growth factor (EGF) ligand activates the EGFR, subsequently activating downstream cell signaling pathways, including the Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, which drives cell proliferation, and promotes cell migration, angiogenesis, and cellular resistance to apoptosis.[26] Cetuximab and panitumumab block the binding of EGF to the EGFR to inhibit its downstream cell proliferative signaling pathways. Mutations in *KRAS* and *NRAS* cause constitutive activation of the Ras/Raf/MAPK signaling pathway and resistance to EGFR inhibition; therefore, these agents are only recommended for *KRAS* and *NRAS* wild-type tumors. The *BRAF* V600E mutation also leads to constitutive activation of Ras/Raf/MAPK pathway and may represent a source of tumor resistance to cetuximab and panitumumab in *KRAS* and *NRAS* wild-type tumors. Severe infusion reactions, including anaphylaxis, have been observed with both cetuximab and panitumumab, although the incidence is lower with panitumumab.[6] Dermatologic reactions, particularly skin rash, can be severe. Other AEs include diarrhea, electrolyte imbalances (magnesium, potassium, calcium), fatigue, ocular toxicities, and interstitial lung disease.[28]

VEGF represents a family of endothelial growth factors that promote angiogenesis by inducing endothelial cell proliferation, migration, permeability, and survival.[26] In the setting of environmental hypoxia, VEGF and other proangiogenic factors are produced by tumor cells and associated stroma, and released into the circulation to stimulate new blood vessels that will support further tumor growth. VEGF binds to VEGF receptors (VEGFR), VEGF receptor-1 and VEGF receptor-2, and VEGF receptor-3, which are expressed on vascular endothelial cells, as well as some cancer cell surfaces.[28] Binding of VEGF to the VEGF receptor activates intracellular signaling pathways that promote angiogenesis. Bevacizumab is a humanized mAb that has a high affinity for binding to circulating soluble VEGF-A, thereby preventing activation of signaling cascades that stimulate angiogenesis.[29] Ziv-aflibercept is a recombinant fusion protein that binds to VEGF-A, VEGF-B, and the angiogenic protein placental growth factor, to inhibit angiogenesis. Ramucirumab, the most recently approved antiangiogenic mAb for colorectal cancer, binds to VEGFR-2. All of these agents can cause infusion-related reactions, hypertension, impaired wound healing, hemorrhage, gastrointestinal perforation, arterial and venous thrombotic events, proteinuria, and reversible posterior leukoencephalopathy syndrome.

Regorafenib is an orally administered small-molecule inhibitor of multiple kinases that regulate normal cellular functions as well as tumor oncogenesis, angiogenesis, and metastasis.[30] AEs associated with regorafenib include diarrhea, hepatotoxicity, and hand-foot syndrome, in addition to those seen with the antiangiogenic mAbs. Regorafenib is a substrate CYP3A4 and, as such, strong CYP3A4 inducers or inhibitors should be avoided during treatment with regorafenib.

Immunotherapy

Pembrolizumab and nivolumab are both programmed cell death-1 (PD-1) blocking antibodies that have received approval by the FDA for use in MSI-H or tumors that are deficient in mismatch repair (dMMR) that has progressed following treatment with a fluoropyrimidine, irinotecan, and oxaliplatin.[6]

Initial Therapy Selection

Considerations for the choice of therapy are based on the goals of therapy, prior therapy(ies), the mutational profile of the tumor, and the toxicity profiles of individual agents. The NCCN does not consider one regimen (ie, oxaliplatin, 5-FU/LV [FOLFOX]; capecitabine plus oxaliplatin [CAPEOX]; irinotecan, 5-FU/LV [FOLFIRI]; infusional 5-FU/LV, capecitabine or oxaliplatin, irinotecan [FOLFOXIRI]) to be preferable over the others as initial therapy, but does recommend one of these regimens, with or without an added targeted therapy, for patients for whom initial therapy with an intensive therapy is appropriate.[6] No biologic agent (ie, bevacizumab, cetuximab, panitumumab, or none) as part of initial therapy is preferred.[6] Targeted therapies can be included in the second-line or third-line treatment of mCRC, depending on prior therapy.[3,6] In patients with advanced or metastatic disease who are not appropriate for intensive therapy, targeted therapies, such as cetuximab or panitumumab (category 2B) are indicated as first-line single-agent treatment by the NCCN guidelines for *KRAS/NRAS* wild-type and left-sided tumors only.[6]

TABLE 4. NCCN Recommendations for Initial Systemic Therapy for Advanced or mCRC[6]

Patient Selection	Treatment Options
Patient appropriate for intensive therapy	• FOLFOX ± bevacizumab • CAPEOX ± bevacizumab • FOLFIRI ± bevacizumab • FOLFOXIRI ± bevacizumab • 5-FU/LV (infusion preferred) ± bevacizumab • Capecitabine ± bevacizumab
	If tumor *KRAS* or *NRAS* wild-type: • FOLFOX + cetuximab OR panitumumab • FOLFIRI + cetuximab OR panitumumab
Patient not appropriate for intensive therapy	• Infusional 5-FU/LV ± bevacizumab • Capecitabine ± bevacizumab
	If tumor *KRAS* or *NRAS* wild-type: • Cetuximab or panitumumab
	If tumor dMMR/MSI-H: • Nivolumab or pembrolizumab

5-FU/LV indicates fluorouracil/leucovorin; CAPEOX, capecitabine/oxaliplatin; dMMR, tumors that are mismatched-repair protein deficient; FOLFIRI, irinotecan, folinic acid, and fluorouracil; FOLFOX, fluorouracil/leucovorin/oxaliplatin; FOLFOXIRI, infusional 5-FU/LV, capecitabine, or oxaliplatin, irinotecan; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability high; NCCN, National Comprehensive Cancer Network; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog.

TABLE 5. NCCN Guidelines for Subsequent Treatment of Advanced or Metastatic Colorectal Cancer[6]

Initial Therapy	Treatment Options
Previous oxaliplatin-containing therapy without irinotecan	• FOLFIRI ± bevacizumab OR ziv-aflibercept OR ramucirumab • Irinotecan ± bevacizumab OR ziv-aflibercept OR ramucirumab
	If tumor *KRAS* or *NRAS* wild-type: • FOLFIRI + cetuximab OR panitumumab • Irinotecan + cetuximab OR panitumumab
	If tumor dMMR/MSI-H: • Nivolumab or pembrolizumab
Previous irinotecan-containing therapy without oxaliplatin	• FOLFOX ± bevacizumab • CAPEOX ± bevacizumab
	If tumor *KRAS* or *NRAS* wild-type: • Irinotecan ± cetuximab OR panitumumab
	If tumor dMMR/MSI-H: • Nivolumab or pembrolizumab
Previous FOLFOXIRI	• Regorafenib • Trifluridine + tipiracil
	If tumor *KRAS* or *NRAS* wild-type: • Irinotecan + cetuximab OR panitumumab
	If tumor dMMR/MSI-H: • Nivolumab or pembrolizumab
Previous fluoropyrimidine without irinotecan or oxaliplatin	• FOLFOX ± bevacizumab • CAPEOX ± bevacizumab • FOLFIRI ± bevacizumab[a] OR ziv-aflibercept OR ramucirumab • Irinotecan ± bevacizumab[a] OR ziv-aflibercept OR ramucirumab • Irinotecan + oxaliplatin ± bevacizumab
	If tumor dMMR/MSI-H: • Nivolumab or pembrolizumab

CAPEOX indicates capecitabine/oxaliplatin; dMMR, tumors that are mismatched-repair protein deficient; FOLFIRI, irinotecan, folinic acid, and fluorouracil; FOLFOX, fluorouracil/leucovorin/oxaliplatin; *KRAS*, Kirsten rat sarcoma viral oncogene homolog; MSI-H, microsatellite instability high; NCCN, National Comprehensive Cancer Network; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog.
[a]Bevacizumab preferred.

The NCCN guidelines for initial systemic therapy for advanced or metastatic CRC treatment are shown in **Table 4**.[6]

Selection of Subsequent Therapy

Patients with wild-type *KRAS/NRAS* tumors who experience progression on therapy that did not contain an EGFR inhibitor should receive cetuximab or panitumumab plus irinotecan, cetuximab, or panitumumab plus FOLFIRI, or single-agent cetuximab or panitumumab. For patients whose therapy did contain an EGFR inhibitor, use of another EGFR inhibitor is not recommended.[6] The NCCN guidelines for subsequent therapy for advanced or metastatic CRC are summarized in **Table 5**.[6]

The NCCN discourages switching to either cetuximab or panitumumab after failure of the other drug.[3] Anti-VEGF therapies are frequently used in cytotoxic drug combinations for the treatment of mCRC. The NCCN panel recommends bevacizumab over ziv-aflibercept and ramucirumab, both of which also block VEGF signaling, for mCRC after progression due to concerns about toxicity, low activity, and costs.[6] Regorafenib, a small-molecule inhibitor of VEGF receptor (VEGFR), is recommended for patients with disease that is refractory to chemotherapy.[6] Trifluridine-tipiracil, a recently approved cytotoxic agent, may be given before or after regorafenib.[6] Pembrolizumab and nivolumab block immune checkpoint proteins and may help in reestablishing immune response against tumors.[31] Either agent may be used in the second or third line, but if tumor progression occurs, then the other agent should

not be offered.[6] Pembrolizumab and nivolumab are recommended for patients whose tumors are dMMR or MSI-H.[6] This information should be routinely obtained during the workup of the patient. Pembrolizumab and nivolumab are also indicated as first line for only dMMR/MSI-H patients with advanced or metastatic disease who are not appropriate for intensive therapy.[6]

Patients with better performance status and fewer comorbidities generally have better outcomes. Surgical resection is considered standard of care for patients with resectable metastatic disease.[6] Many patients who present with mCRC have unresectable metastases. Some patients with metastatic disease may be candidates for neoadjuvant (preoperative) combination chemotherapy in an effort to reduce the size of metastases, thereby converting the

patient to having resectable disease. Any active therapy may be used to convert the patient to having resectable disease, although it is important to keep in mind that irinotecan- and oxaliplatin-based chemotherapeutic regimens can cause liver damage.[6] The addition of a targeted biologic agent (eg, cetuximab or panitumumab for *KRAS/NRAS* wild-type tumors or bevacizumab) to chemotherapy can increase tumor response and complete resection rates, and is considered acceptable.[6] Surgery is recommended as soon as possible after the patient is deemed resectable to avoid chemotherapy-induced hepatic injury.[6] Adjuvant therapy after metastasectomy is recommended.[6]

New and Emerging Agents for mCRC

In the United States, several novel agents have been approved for mCRC since 2015. These agents include the antiangiogenic agent ramucirumab, cytotoxic therapies such as trifluridine-tipiracil (TAS-102), and the immune checkpoint inhibitors pembrolizumab and nivolumab.[31-34]

Ramucirumab

Ramucirumab is a vascular endothelial growth factor receptor 2 (VEGFR2) antagonist that is administered as an intravenous infusion.[31] In contrast to bevacizumab and ziv-aflibercept, which bind to soluble VEGF-A, ramucirumab binds to the extracellular domain of VEGFR2, thereby inhibiting the binding of several VEGF ligands and interrupting the VEGF-VEGFR signaling pathway at the receptor level.[35] Ramucirumab has been approved in combination with FOLFIRI for patients with mCRC who have disease progression on or after prior therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine.[31] The efficacy and safety of ramucirumab versus placebo was assessed in the RAISE phase 3 clinical trial with a combination with second-line FOLFIRI in patients experiencing mCRC progression during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine.[35] A total of 1072 patients were enrolled. Median overall survival (OS) was 13.3 months versus 11.7 months for patients in the ramucirumab group versus the placebo group, respectively (*P* = .022). Grade 3 or higher AEs were seen in greater than 5% of patients and included neutropenia (38% vs 23% in the ramucirumab vs placebo groups, respectively), with incidence of febrile neutropenia (3% vs 2%), hypertension (11% vs 3%), diarrhea (11% vs 10%), and fatigue (12% vs 8%). A subgroup analysis of the phase 3 RAISE clinical trial demonstrated a statistically significant improvement in OS, regardless of time to disease progression or *KRAS* status.[31,36]

Ramucirumab is FDA approved in combination with FOLFIRI for the treatment of mCRC with disease progression on or after prior therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine.[31] Ramucirumab is indicated as one of the options in the NCCN clinical guidelines for the primary treatment of unresectable metachronous

metastases in patients who have had prior adjuvant FOLFOX/CAPEOX within the past 12 months, or as subsequent systemic therapy for patients who received adjuvant FOLFOX/CAPEOX more than 12 months prior or received previous 5-FU/leucovorin or capecitabine.[6] The ESMO guidelines recommend ramucirumab as a second-line agent in combination with FOLFIRI in patients with disease progression during or after first-line therapy with oxaliplatin, bevacizumab, and a fluoropyrimidine.[3] Although ramucirumab has no contraindications, it does have a boxed warning for increased risk of hemorrhage, gastrointestinal (GI) perforation, and impaired wound healing.[31] Patients should permanently discontinue ramucirumab if they experience severe bleeding or GI perforation. Patients who experience impaired wound healing should discontinue ramucirumab until the wound is fully healed.[31]

Trifluridine-tipiracil (TAS-102)

Trifluridine-tipiracil is an oral drug combination of trifluridine, a cytotoxic thymidine analog, and tipiracil hydrochloride, a thymidine phosphorylase inhibitor.[6] Trifluridine is incorporated into DNA, thereby interfering with DNA synthesis and inhibiting cell proliferation. Tipiracil prevents the rapid degradation of trifluridine via thymidine phosphorylase. Results from the RECOURSE phase 3 clinical trial were critical for FDA approval of this novel cytotoxic drug. The trial enrolled 800 patients with mCRC who had progressed beyond 2 prior regimens. Patients were randomized 2:1 to receive trifluridine-tipiracil or placebo. The median OS improved from 5.3 months with placebo to 7.1 months with TAS-102 (hazard ratio [HR] .68; 95% CI, 0.58-0.81; *P* < .001).[29] Trifluridine-tipiracil is FDA approved for the treatment of mCRC in patients who have previously received fluoropyrimidine-, oxaliplatin-, and irinotecan-based chemotherapy, an anti-VEGF mAb, and if *RAS* wild-type, an anti-EGFR therapy.[3] The NCCN recommendations include trifluridine-tipiracil as a third-line treatment option for patients with disease progression through standard therapies.[6] Trifluridine-tipiracil can be given prior to or following treatment with regorafenib as there are no data to suggest the best order for these agents.[6] The most frequently reported adverse reactions with trifluridine and tipiracil include asthenia/fatigue (52%), nausea (48%), decreased appetite (39%), diarrhea (32%), vomiting (28%), and infections (27%). Dose delays and reduced doses may be necessary to reduce incidence and severity of neutropenia and thrombocytopenia.[32,37]

Immunotherapies: Anti–PD-1/PD-L1 Monoclonal Antibodies

The immune system includes multiple checkpoints that turn off or prevent inappropriate immune activity.[38] Tumor cells manipulate these mechanisms, thereby reducing T cell activity within tumors and escaping immunosurveillance. The PD-1/PD-L1 system is often used by tumors to block T cell activity. PD-1 is a protein on

T cells which, when bound to PD-L1, a protein present on normal and malignant cells, inhibits T cell activity. Increased PD-1/PD-L1 expression is increased in a subset of mCRC and in association with MSI.[39] Coupled with the very high mutation prevalence in, and high antigenic potential of CRC, the therapeutic efficacy of immune checkpoint inhibitors has been investigated. Five anti–PD-1/PD-L1 mAbs have received FDA approval and are currently under study: pembrolizumab, nivolumab, atezolizumab, durvalumab, and avelumab.[40]

Pembrolizumab and nivolumab were FDA approved in 2017 for the treatment of adult and pediatric patients with dMMR and MSI-H mCRC that has progressed following treatment with a fluoropyrimidine, oxaliplatin, and irinotecan.[33,34] Approval for each was by the accelerated pathway and continued approval is predicated on future trial results. The CheckMate 142 trial is evaluating nivolumab in 74 patients with MSI-H mCRC who received either single-agent nivolumab or nivolumab plus ipilimumab. Early results indicate that 31.3% of patients receiving nivolumab responded. Responses to nivolumab were seen in patients regardless of tumor PD-L1 expression, *KRAS/BRAF* mutation status, or clinical history (ie, Lynch syndrome).[17,40] Nivolumab demonstrated durable responses as well as disease control in patients with dMMR/MSI-H mCRC and the treatment was well tolerated with no new safety issues.[17,41] The trial is ongoing.[17,41] For pembrolizumab, several small trial results were pooled, for a total of 149 patients with MSI-H cancers, of which 90 were CRC.[40,42] Among the 90 patients with CRC, the objective response rate (ORR) was 36% (95 CI, 26%-46%) and lasted from 1.6 to 22.7 months.[40,42]

Atezolizumab targets PD-L1 and has been approved for previously treated non-small cell lung cancer and locally advanced or metastatic urothelial carcinoma.[43] Atezolizumab is currently in several clinical trials for colorectal cancer in combination with targeted or chemotherapy regimens. Researchers of phase 3 clinical trials are investigating atezolizumab as a third-line treatment for mCRC as monotherapy or in combination with cobimetinib.[44] Tremelimumab is in phase 1 (NCT03005002, NCT02754856, NCT03202758) and phase 2 (NCT03007407, NCT03122509, NCT03202758, NCT02870920, NCT02888743) clinical trials in combination with durvalumab for mCRC. Durvalumab targets PD-L1 and tremelimumab targets CTLA-4.[44,45] Results presented at the 2018 Gastrointestinal Cancers Symposium demonstrated that in the phase 2 CheckMate 142 trial, the combination of nivolumab plus ipilimumab in patients with dMMR or MSI-H mCRC provided durable clinical benefits. The ORR was 55% at 12.4 months and the rate of disease control that persisted longer than 12 weeks was 80%.[46]

Pharmacists must be aware that the checkpoint inhibitors are associated with unique immune-related adverse effects and toxicities. Healthcare professionals who treat these patients must be able to adequately address the individual toxicities and provide patient management and care considerations. Additionally, immunotherapy takes longer to elicit responses as compared with chemotherapy. Therefore, patients may have stable disease or disease progression after initial treatment and before they observe clinical improvement.[47] **Table 6** lists the common immune-related toxicities and outlines an appropriate management approach.[47]

Additional Emerging Targeted and Immunotherapy Therapies

Other emerging targeted therapies include BRAF inhibitors (dabrafenib, vemurafenib, encorafenib), anti-fibroblast growth factor receptor (FGFR) agents (ponatinib, BGJ398), anti-RET agents (ponatinib, cabozantinib, vandetanib, apatinib, ponatinib, RXDX-105, sunitinib, sorafenib), anti-HER2 agents (sapitinib, neratinib, HER2 vaccine, trastuzumab, pertuzumab, lapatinib, tucatinib), and anti-hepatocyte growth factor receptor (HGFR) and the ligand c-MET (crizotinib, tivantinib, cabozantinib, INC280, AMG102, AV299).[35,48]

TABLE 6. Management of Immune-Related Toxicities Associated with Checkpoint Inhibitors[47]

Common Adverse Events	Workup for Alternative/ Noninflammatory Etiologies	Grade of Toxicity	Recommended Management of Immune-Mediated Adverse Events
Gastrointestinal Diarrhea/Colitis	Rule out infectious etiology (*Clostridium difficile*)	Mild	• Symptom management • Consider budesonide 9 mg daily • Continue immunotherapy
		Moderate	• Delay immunotherapy • Methylprednisolone IV or oral equivalent 0.5-1 mg/kg/day • Consider GI consult and colonoscopy • When improves to grade 1 or less, taper steroids over at least 4 weeks
		Severe	• Discontinue immunotherapy • Methylprednisolone IV 1-2 mg/kg/day • When improves to grade 1 or less, taper steroids over at least 4 weeks • No improvement in symptoms within 48-72 hours, consider second-line immunosuppression (infliximab)

(continued)

TABLE 6. *(continued)* Management of Immune-Related Toxicities Associated with Checkpoint Inhibitors[47]

Common Adverse Events	Workup for Alternative/ Noninflammatory Etiologies	Grade of Toxicity	Recommended Management of Immune-Mediated Adverse Events
Hepatitis	Evaluate for • Alcohol intake • Concomitant medications with hepatotoxic potential • Rule out biliary disease/ obstruction	Mild	• Continue immunotherapy • Repeat LFTs in 1 week
		Moderate	• Delay immunotherapy • Repeat LFTs every 3-5 days • Methylprednisolone 0.5-1 mg/kg/day or oral equivalent • Monitor LFTs every 3 days. When improves to mild or baseline, taper steroids over at least 4 weeks
		Severe	• Discontinue therapy • Increase frequency of LFT monitoring to 1-2 days • Methylprednisolone IV 1-2 mg/kg/day • Consult GI • No improvement in 48-72 h, consider second-line immunosuppression
Pneumonitis	Evaluate for • Pulmonary embolism • Cardiac causes • Infectious etiology • COPD • Seasonal allergies/cough from postnasal drip	Mild	• Delay immunotherapy • Monitor for symptoms • Repeat chest radiograph in 2-4 weeks
		Moderate	• Delay immunotherapy • Monitor symptoms closely, consider hospitalization • Re-image every 1-3 days • Pulmonary and ID consults, consider bronchoscopy • Methylprednisolone IV or oral equivalent 1-2 mg/kg/day • When symptoms improve, taper steroids over at least 4 weeks
		Severe	• Discontinue immunotherapy • Methylprednisolone IV 2-4 mg/kg/day, taper steroids over at least 6 weeks • No improvement in symptoms, consider second-line immunosuppression (infliximab, mycophenolate mofetil, IVIG)
Dermatologic toxicities	Rule out noninflammatory causes (allergic reaction to other medications, photosensitivity, etc)	Mild	• Continue immunotherapy • Supportive management: emollients, low potency topical steroids, antihistamines
		Moderate	• Continue immunotherapy • Moderate-high potency topical steroids • If persistent despite optimal topical management, consider methylprednisolone 0.5-1 mg/kg/day or oral equivalent • If improved to mild or resolves, taper steroids over 4 weeks • Consider dermatology evaluation and skin biopsy
		Severe	• Delay immunotherapy • Methylprednisolone IV 1-2 mg/kg/day or oral equivalent • If improves to mild or resolves, taper steroids over at least 4 weeks • Consider skin biopsy
Endocrinopathy	Rule out noninflammatory etiology of symptoms	Mild	• Continue immunotherapy • If abnormal TSH, add free T4 and T3 • Consider morning cortisol, ACTH
		Moderate	• TSH, free T4, morning cortisol, ACTH • Consider pituitary MRI • Methylprednisolone 1-2 mg/kg/day or oral equivalent • If improved, taper steroids over at least 4 weeks • Hormone replacement therapy if indicated • Endocrine consult
		Severe	• Delay or discontinue immunotherapy • Concern for adrenal crisis: rule out infection/sepsis, blood pressure support • Stress doses of mineralocorticoid

ACTH indicates adrenocorticotropic hormone; COPD, chronic obstructive pulmonary disease; GI, gastrointestinal; ID, infectious disease; IV, intravenous; IVIG, intravenous immunoglobulin; LFT, liver function test; MRI, magnetic resonance imaging; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.
Reprinted with permission from Wolters Kluwer Medknow Publications. Dine J, Gordon R, Shames Y, Kasler MK, Barton-Burke M. Immune checkpoint inhibitors: an innovation in immunotherapy for the treatment and management of patients with cancer. *Asia Pac J Oncol Nurs*. 2017;4(2):127-135.

TABLE 7. Promising Agents for mCRC in Clinical Trials[44]

Agent	Therapeutic Class	Targeted Indication	Anticipated US Launch Date
Molecular-Targeted Agents			
Encorafenib (LGX-818) + binimetinib (MEK-162)	BRAF inhibitor/MEK1/2 inhibitor	Second-line treatment in *BRAF*-mutant mCRC in combination with cetuximab	2020
Masitinib (AB-1010)	Tyrosine kinase inhibitor of c-Kit, PDGFR, FGFR3	Second-line treatment for patients with mCRC progression after standard chemotherapy	2019
Napabucasin (BBI-608)	Small-molecule cancer stem cell stemness inhibitor targeting STAT3	Second-line treatment in combination with FOLFIRI regimen (fluorouracil, leucovorin, and irinotecan), with or without bevacizumab	2020
Immunotherapies			
Atezolizumab (Tecentriq)	Anti–PD-L1 monoclonal antibody	Third-line treatment as monotherapy or in combination with cobimetinib	2020
Avelumab (Bavencio)	Anti–PD-L1 monoclonal antibody	Monotherapy and in combination with cetuximab and FOLFOX as first-line therapy for mCRC	N/A
Pembrolizumab (Keytruda)	Anti–PD-1 monoclonal antibody	First-, second-, and third-line treatment for MSI-H or dMMR mCRC	Second- and third-line: 2017 First-line: 2020

BRAF indicates V-raf murine sarcoma viral oncogene homolog B1; dMMR, tumors that are mismatched-repair protein deficient; FGFR3, fibroblast growth factor receptor 3; FOLFIRI, irinotecan, folinic acid, and fluorouracil; FOLFOX, fluorouracil/leucovorin/oxaliplatin; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability-high; PD-1, programmed death receptor 1; PD-L1, programmed death ligand-1; PDGFR, platelet-derived growth factor receptor.
Reprinted with permission from MediMedia Managed Markets. Fellner C. Promising drugs in clinical development to treat advanced colorectal cancer. *P T.* 2017;42(4):262-265.

Most are being explored as single agents, but some are in trials as a component of combination therapies. **Table 7** from Fellner 2017 outlines some of the promising drugs in development for mCRC.[44]

Both molecularly targeted agents such as encorafenib (LGX-818) + binimetinib (MEK-162), masitinib (AB-1010), napabucasin (BBI-608) as well as immunotherapies, such as atezolizumab and pembrolizumab, are being investigated as first-, second-, or third-line treatments (see Table 2).[44] BRAF and MEK protein kinases are key in the MAPK signaling pathway. Encorafenib is an oral small-molecule selective BRAF inhibitor that is being investigated in combination with binimetinib, an oral small-molecule inhibitor of MEK1/2 for the second-line treatment of patients with *BRAF*-mutant mCRC. It is coadministered with cetuximab.[44] Masitinib is an oral phenylaminothiazole-type tyrosine kinase inhibitor (TKI). It targets both the wild-type and mutated forms of c-Kit (stem cell factor receptor), platelet-derived growth factor receptor (PDGFR) alpha/beta, Lyn tyrosine kinase, and fibroblast growth factor receptor 3 (FGFR3).[44,49] Napabucasin is an oral cancer agent that inhibits cancer stemness pathways that allow cancer stem cells to self-renew and differentiate into heterogenous cancer cells. It targets signal transducer and activator of transcription 3 (STAT3).[44]

Genomics-Driven Therapeutic Approaches

As next-generation sequencing of refractory tumors to inform therapeutic decision making outside of clinical trials continues, prescribers may identify actionable molecular alterations to support on- or off-label use of potentially beneficial targeted treatments. Common actionable targets include mutations in *KRAS*, *CDKN2A/B*, *PIK3CA*, *FGFR*, *PTEN/AKT*, and *HER2*, among others.[50] Although the overall value of this approach remains unproven, reports and anecdotal experiences of patients benefiting from genomic-driven targeted therapy are encouraging, and off-label use of anticancer agents in this setting will likely continue to increase.[51-55]

Conclusions

Patients with unresectable mCRC remain incurable, with unsatisfactory survival rates, indicating a critical need to improve therapeutic outcomes. Recent advances in targeted therapy and immunotherapy represent meaningful progress in treatment strategies for advanced and mCRC; clinicians need to be familiar with genetic biomarkers that identify patients who are appropriate candidates for specific therapies. Progress in classifying CRC based on clinical and molecular features has led to a molecular subtype algorithm that may inform treatment decisions but is currently not recommended for clinical practice. Most recently, the angiogenesis inhibitor ramucirumab; trifluridine-tipiracil, a novel oral cytotoxic inhibitor of cell growth and proliferation; and PD-1 inhibitors nivolumab and pembrolizumab have demonstrated improved survival in selected settings and are now approved for patients with mCRC. The wide array of molecular alterations in mCRC has provided multiple therapeutic targets against which numerous emerging targeted therapies are currently in development. ∎

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