The Emergence of Targetable Pathways in Colorectal Cancer

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Abstract: Colorectal cancer continues to be one of the leading causes of cancer-related morbidity and mortality globally. Despite an overall decreasing incidence of the disease, early-onset colorectal cancer is a growing concern. Fluoropyrimidine-based doublet chemotherapy has remained the backbone of treatment in the metastatic setting during the past 2 decades. The increasing accessibility and decreasing cost of molecular profiling have made it possible to acquire further insight into prognostic and predictive biomarkers that ultimately help physicians to provide precision medicine in the clinic. In this review, we describe a contemporary biomarker-driven approach to first-line and subsequent-line therapies and highlight the important molecular alterations that affect the treatment of advanced colorectal cancer, along with the supporting clinical trial data.

Keywords
Biomarkers, ctDNA, metastatic colorectal cancer, molecular profiling, next-generation sequencing

Introduction

Colorectal cancer (CRC) is the third most common cancer in the United States, with a projected 150,000 new cases and more than 55,000 deaths in 2021.[1] Globally, CRC makes up 10% of all new cancer cases and is the second-leading cause of cancer-associated mortality.[2] Although the risk for CRC increases with age, more than 10% of cases are defined as early-onset CRC, in which patients are younger than 50 years at diagnosis. The proportion of patients with early-onset disease continues to increase with time.[3,4] Distinct clinical and molecular differences are observed in the group with early-onset CRC, including fewer adenomatous polyposis coli (*APC*) gene mutations, higher rates of signet ring histology, and higher rates of consensus molecular subtype 1 (CMS1).[5] Despite declines in overall incidence over the past 4 decades, attributed mainly to risk reduction and uptake of screening colonoscopies, 20% to 25% of patients present with synchronous metastases, with a 5-year overall survival rate of 14%.[4] In the metastatic setting (mCRC), systemic treatment historically has consisted of fluoropyrimidine-based combination regimens with the addition of biologic agents. However, with the emergence and increasing availability of molecular profiling, the field is moving toward a personalized biomarker-driven approach. In this review,

we focus on highlighting targetable molecular pathways in the mCRC setting. The overall biomarker-selective approach to first-line therapy and beyond is summarized in the Figure, and ongoing clinical trial efforts are summarized in the Table.

Mismatch Repair–Deficient/Microsatellite Instability–High Tumors

DNA mismatch repair (MMR) is an imperative corrective mechanism that maintains genomic stability by mending single base pair insertions or deletions, which are generated during DNA replication owing to slippage by DNA polymerases.[6] Relevant MMR genes include *MLH1*, *MSH2*, *MSH6*, *PMS1*, and *PMS2*.[7] Germline mutations in one of the MMR genes result in short, repetitive DNA sequences termed microsatellites, and tumors that exhibit a high level of microsatellites, as seen in hereditary nonpolyposis colorectal cancer (HNPCC), are designated microsatellite instability–high (MSI-H).[8,9] Some sporadic cases of CRC demonstrate loss of MMR protein expression via an epigenetic mechanism, hypermethylation of the *MLH1* promoter region, which also results in an MSI-H phenotype.[10,11] Overall, MMR-deficient (dMMR)/MSI-H tumors make up 15% of all cases of CRC[12]; a higher incidence is seen in early stages (21% in stage II, 14% in stage III) than in metastatic disease (5% in stage IV).[13,14] Notably, dMMR/MSI-H status is a predictive and prognostic biomarker in stage II CRC. It confers a lack of benefit to adjuvant fluoropyrimidine monotherapy and is associated with an improved overall prognosis.[15-17]

In a proof-of-concept study, Le and colleagues demonstrated that dMMR/MSI-H status is a robust biomarker for response to pembrolizumab (Keytruda, Merck) in treatment-refractory MSI-H tumors; pembrolizumab treatment achieved an objective response rate (ORR) of 40% (4/10 patients) in the dMMR CRC cohort, compared with 0% (0/18 patients) in the MMR-proficient (pMMR) group.[18] In a follow-up study that enrolled 86 patients with 12 different tumor histologies, treatment with pembrolizumab led to an ORR of 53% for all patients with dMMR tumors and of 52% for those with dMMR CRC tumors.[19] The use of pembrolizumab was further supported in a larger cohort in KEYNOTE-164, with an ORR of 33%.[20] The phase 2 multicohort CheckMate 142 study included a cohort in which nivolumab (Opdivo, Bristol Myers Squibb) was evaluated in the second-line and beyond setting. Patients with recurrent or metastatic dMMR/MSI-H CRC were given nivolumab at 3 mg/kg every 2 weeks; the ORR was 31%, and the disease control rate was 69%.[21] In another cohort of CheckMate 142, nivolumab at 3 mg/kg and low-dose ipilimumab (Yervoy, Bristol Myers Squibb) at 1 mg/kg every 3 weeks were administered for 4 cycles, followed by maintenance nivolumab at 3 mg/kg every 2 weeks.[22] In recent data from the European Society for Medical Oncology (ESMO) World Congress on Gastrointestinal Cancer 2021, the ORR was 65%, and median progression-free survival (PFS) and overall survival (OS) had not been reached after a median follow up of 50.9 months.[23] Of note, 12% of patients had disease progression as best response.

In the first-line setting, the role of immunotherapy has now been established on the basis of the pivotal phase 3 KEYNOTE-177 trial, which compared pembrolizumab with chemotherapy. Chemotherapy consisted of 5-fluorouracil (5-FU), oxaliplatin, and leucovorin (FOLFOX) or 5-FU, irinotecan, and leucovorin (FOLFIRI), with or without bevacizumab or cetuximab (Erbitux, Lilly). Pembrolizumab was superior to chemotherapy, associated with a doubling of PFS (16.5 vs 8.2 months; hazard ratio [HR], 0.60; P=.0002).[24] However, 30% of the patients in the pembrolizumab group had progression as best response, compared with 12% in the chemotherapy group. Potential explanations of the resistance to immunotherapy include low tumor mutation burden, presence of *JAK* mutations, loss of beta$_2$-microglobulin, and pseudo-progression.[25-27] These percentages contrast with the lower rate of disease progression as best response (12%) observed with nivolumab and ipilimumab in CheckMate 142 in the previously treated setting; one possible explanation is that clonal evolution and a higher tumor mutational burden (TMB) may develop in heavily pretreated patients, which can be predictive of immunotherapy response.[25] However, cross-trial comparison is further limited by single-agent vs dual immune checkpoint blockade. In the recent update presented at the 2021 American Society of Clinical Oncology (ASCO) Annual Meeting, median OS was not reached vs 36.7 months (HR, 0.74; P=.0359), favoring pembrolizumab, but the difference did not meet statistical significance.[28] The high crossover rate of 60% likely contributed to this finding. In another cohort of CheckMate 142, dual immune checkpoint inhibition with nivolumab at 3 mg/kg every 2 weeks and low-dose ipilimumab at 1 mg/kg every 6 weeks was administered in the first-line setting. Longer-term data are awaited; the 15-month OS rate was 84%, and the centrally assessed ORR was 58% after a median follow-up of 19.9 months.[29]

Pembrolizumab is approved by the US Food and Drug Administration for first-line therapy and is the preferred option in the National Comprehensive Cancer Network (NCCN) guidelines; nivolumab/ipilimumab is also approved as an alternative option on the basis of CheckMate 142 data.[30] It remains unanswered if dual checkpoint inhibition will provide further benefit in

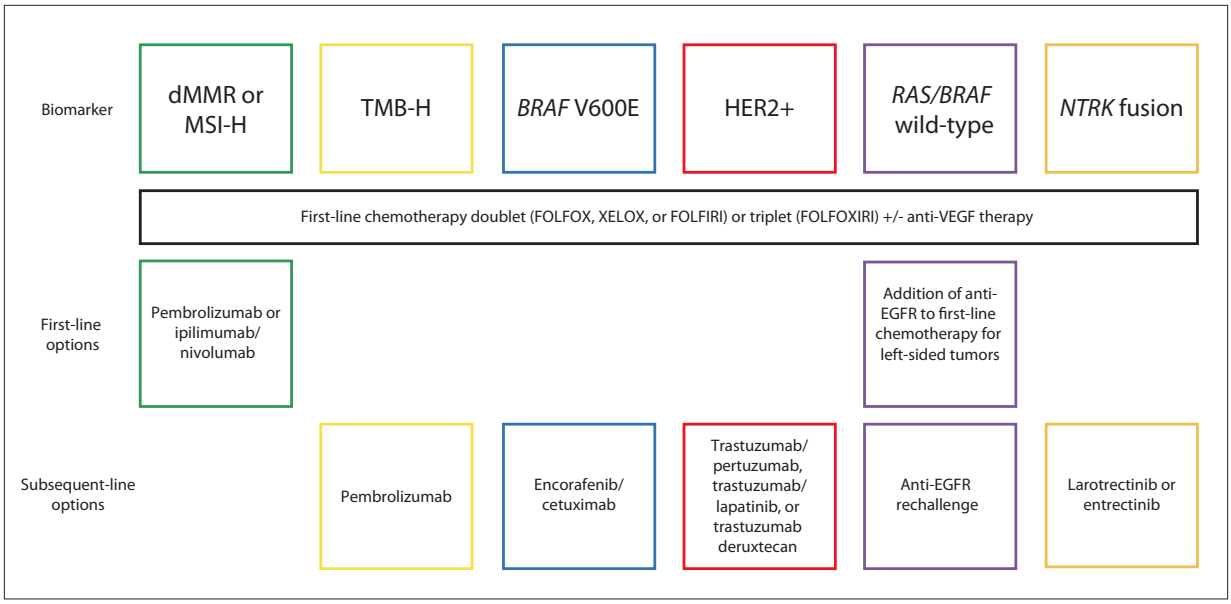

Figure. Schematic of a biomarker-driven approach to first-line and subsequent-line therapies in the treatment of metastatic colorectal cancer.

dMMR, mismatch repair–deficient; EGFR, endothelial growth factor receptor; FOLFIRI, 5-fluorouracil, irinotecan, and leucovorin; FOLFOX, 5-fluorouracil, oxaliplatin, and leucovorin; FOLFOXIRI, 5-fluorouracil, oxaliplatin, irinotecan, and leucovorin; MSI-H, microsatellite instability–high; HER2+, positive for human epidermal growth factor receptor 2 overexpression; TMB-H, tumor mutational burden–high; VEGF, vascular endothelial growth factor; XELOX, oxaliplatin plus capecitabine.

comparison with anti–programmed death 1 (PD-1) monotherapy. The ongoing first-line COMMIT study is comparing combination chemotherapy plus atezolizumab (Tecentriq, Genentech) with atezolizumab alone, to see if intensification with chemotherapy will further improve outcomes.[31] Another unanswered area is the role of combination immunotherapy with either anti–vascular endothelial growth factor (VEGF) or anti–epidermal growth factor receptor (EGFR) therapy, according to tumor sidedness. The administration of atezolizumab and bevacizumab together was shown to have clinical activity; the disease control rate was 90% in patients with heavily pretreated mCRC,[32] warranting potential further studies in this space. The synergistic effect of anti-VEGF and immune checkpoint inhibition has been demonstrated in other tumors, such as hepatocellular carcinoma; this can be attributed to blockage of the VEGF effects of mobilization and proliferation of regulatory T cells, and the release of immunosuppressive cytokines.[33]

Tumor Mutation Burden–High Tumors

TMB is a measurement of the number of non-synonymous somatic mutations identified per megabase of the genome, and it is an emerging biomarker for predicting response to immunotherapy.[34,35] An increased TMB leads to the formation of neoantigens, which in turn are more likely to trigger an antitumor immune response.[35,36] Pembrolizumab carries another tumor-agnostic indication for TMB-high (TMB-H) tumors, which are defined as tumors with more than 10 mutations per megabase on the FoundationOne CDx companion assay. MSI-H and TMB-H tumors show significant overlap; in general, MSI-H tumors are a subset of TMB-H tumors; 97% of MSI-H tumors were demonstrated to have a TMB of at least 10 mutations per megabase in a study of 100,000 cancers comprising more than 100 types of malignancies.[37] However, only 16% of the TMB-H cases were MSI-H. In a study of CRCs (n=6004), 3% of the microsatellite stable (MSS) tumors were found to be TMB-H, defined as 11.7 mutations per megabase.[38] Within the MSS group, TMB-H tumors were more likely to harbor mutations in other DNA proofreading genes, including *POLE*. Therefore, a distinct population of patients with pMMR/MSS/TMB-H tumors exists who may be candidates for immunotherapy.

Data to support pembrolizumab in TMB-H tumors stems from the phase 2 KEYNOTE-158 study, which enrolled patients with multiple tumor types. In the overall study population, the ORR was 29% in the TMB-H cohort (13% of all patients) and 6% in the non–TMB-H group.[39] In the published biomarker analysis, patients

with mCRC were notably not included as a cohort, but the tumor-agnostic approval allows the use of pembrolizumab in mCRC. The TAPUR basket study included a pMMR/MSS/TMB-H (defined as 9 mutations per megabase) mCRC cohort in which patients were treated with pembrolizumab. In a preliminary report at the 2020 ASCO Gastrointestinal Cancers Symposium, 4% (1/27) of patients had a partial response and 28% (7/27) patients had disease control.[40] The threshold to label a tumor TMB-H varies between studies; thus, consensus and standardization will be required in the future. In addition, the optimal threshold for mCRC remains unknown, but it may be higher, as suggested by Schrock and colleagues.[41]

BRAF V600E and Atypical *BRAF* Mutations

BRAF is a serine/threonine protein kinase involved in the mitogen-activated protein kinase (MAPK) pathway, which consists of the RAS/RAF/MEK/ERK signaling cascade in the transduction of growth signals to cellular responses.[42,43] Oncogenic *BRAF* mutations lead to RAS-independent MAPK pathway activation and uncontrolled cell growth.[44] The incidence of *BRAF* V600E activating mutations in CRC is approximately 12.5% in all stages and 10% in stage IV,[45,46] and these mutations are strong prognostic predictors of poor outcomes in both early and metastatic disease.[47-49] However, for *BRAF* V600E–mutated/MSI-H tumors, the mutation does not carry the same adverse prognostic effect in this setting.[50,51] *BRAF* V600E is also predictive of a lack of benefit from anti-EGFR agents, as demonstrated by 2 large meta-analyses.[52,53]

The landmark BEACON study enrolled patients with *BRAF* V600E–mutated tumors who had received 1 or 2 prior regimens. Patients were randomly assigned to one of the following 3 regimens: triplet therapy (encorafenib [Braftovi, Array BioPharma], binimetinib [Mektovi, Array BioPharma], and cetuximab); doublet therapy (encorafenib and cetuximab); or control (investigator's choice of chemotherapy). In the initial report, a survival benefit was observed in a comparison of the triplet therapy group with the control group, with a near doubling of OS (median OS [mOS], 9.0 vs 5.4 months, respectively; HR, 0.52; $P<.01$).[54] The doublet therapy group also showed improved survival in comparison with the control group, with an mOS of 8.4 months (HR, 0.60; $P<.001$). In a subsequent update, mOS was 9.3 months for both the triplet and the doublet groups (HR, 0.60 for triplet vs control; HR, 0.61 for doublet vs control).[55] These results established encorafenib and cetuximab as the standard of care in the second-line setting. In a post hoc analysis, prior bevacizumab use did not appear to affect OS in the doublet therapy arm but was associated with worse outcomes in the triplet therapy arm.[56] It has been suggested previously that resistance to anti-EGFR therapy can lead to increased VEGF expression and potential sensitization to anti-VEGF therapy.[57] In contrast, resistance to anti-VEGF therapy may lead to subsequent resistance to anti-EGFR therapy.[58] Further data are needed, and prior bevacizumab treatment does not affect the use of encorafenib and cetuximab at this time.

In the first-line setting, the phase 2 ANCHOR-CRC study met its primary endpoint, with an ORR of 47.8%. Median PFS and OS were 5.8 and 17.2 months, respectively.[59] The ongoing BREAKWATER study is a global multicenter phase 3 trial enrolling patients with *BRAF* V600E–mutated tumors and randomizing them to doublet therapy (encorafenib and cetuximab), doublet therapy plus chemotherapy, or control (investigator's choice of chemotherapy).[60]

With the emergence of next-generation sequencing (NGS), non-V600E mutations are increasingly being identified. These atypical *BRAF* mutations make up about 20% to 25% of all *BRAF* mutations, and the overall incidence in mCRC is approximately 2%.[61] *BRAF* mutations are classified into 3 groups: class 1 (V600E, RAS-independent), class 2 (codons 597/601, RAS-independent), and class 3 (codons 594/596, RAS-dependent with impaired kinase activity).[62] Data on the prognostic and predictive implications of atypical *BRAF* compared with *BRAF* V600E mutations are mixed. In a retrospective multicohort study, tumors with non-V600E mutations were associated with improved survival compared with V600E and wild-type tumors (mOS, 60.7 vs 11.4 vs 43.0 months, respectively; $P<.001$), and this benefit persisted on multivariate analysis (HR, 0.18).[61] Tumors with non-V600E mutations are less likely to be high-grade, to be right-sided, or to involve the peritoneum.[61,63] In contrast, a European analysis demonstrated shorter OS and PFS in patients with atypical *BRAF* mutations than in those with *BRAF* wild-type mutations.[64] The effect of atypical *BRAF* mutations on response to anti-EGFR therapy was studied by Yaeger and colleagues. Class 2 mutations were found in 30% of patients, and 70% had class 3 mutations; the proportion of patients who responded to anti-EGFR therapy in the first or second line was 17% for class 2 and 78% for class 3.[65] In a separate study, no responses to anti-EGFR therapy were reported, and 55% of the patients (6/11) had stable disease.[63] Novel therapeutic approaches are needed; the ERK 1/2 inhibitor ulixertinib is under investigation in the BVD-523-ABC study, which includes a cohort with atypical *BRAF*–mutated mCRC.[66]

HER2 Overexpression

The *ERBB2* gene codes for the ErbB2 protein (also known as HER2/neu), a plasma membrane–bound receptor

Table. Selected Ongoing Biomarker-Driven Clinical Trials

Biomarker Approach	Trial	Setting	Intervention
dMMR/MSI-H	COMMIT	First-line	Atezolizumab + chemotherapy vs atezolizumab
BRAF V600E mutations	BREAKWATER	First-line	Encorafenib + cetuximab + chemo vs encorafenib + cetuximab vs chemotherapy
Atypical *BRAF* mutations	BVD-523-ABC	Refractory	Ulixertinib
HER2 overexpression	DESTINY-CRC02	Refractory	Trastuzumab deruxtecan at 2 different dosing levels
RAS/BRAF wild-type for anti-EGFR rechallenge	NCT04616183	Refractory	Cetuximab + abemaciclib + LY3214996
KRAS G12C mutation	KRYSTAL-10	Second-line	Adagrasib + cetuximab
NTRK fusions	TRIDENT-1	Refractory	Repotrectinib
	NCT03215511	Refractory	Selitrectinib

dMMR, mismatch repair–deficient; HER2, human epidermal growth factor receptor 2; MSI-H, microsatellite instability–high.

tyrosine kinase that can form heterodimers with any of the other ErbB family tyrosine kinases, leading to the activation of downstream signaling pathways.[67] Testing can be performed through immunohistochemistry (IHC) staining for the human epidermal growth factor receptor 2 (HER2) protein, in situ hybridization (ISH) for gene amplification, or reverse transcription polymerase chain reaction for overexpression of HER2 RNA.[68] Approximately 2% to 5% of CRCs overexpress HER2,[69-71] and this finding is enriched in the *KRAS*/*BRAF* wild-type population.[72]

HER2-directed therapy can be considered in patients whose disease has progressed on oxaliplatin- and/or irinotecan-based chemotherapy, or in an earlier setting in patients who are not candidates for intensive chemotherapy regimens.[30] In the HERACLES phase 2 trial, patients with refractory mCRC who had *KRAS* exon 2 wild-type, HER2-overexpressing tumors were administered weekly trastuzumab (a monoclonal antibody against HER2) and daily lapatinib (a small-molecule inhibitor of EGFR and HER2 receptors). Of the screened patients, 5% had HER2-positive tumors. Among these HER2-positive patients, the ORR was 30% (8/27), a complete response (CR) occurred in 1 patient, and 44% of patients (12/27) had stable disease.[71] However, one-fifth of patients experienced central nervous system (CNS) disease progression, which may reflect the underlying CNS tropism of HER2-positive tumors,[73] similar to what is observed in breast cancer. Baseline CNS imaging should be considered for patients who have CRC with HER2 overexpression, and special attention should be paid to any neurologic symptoms. The MOUNTAINEER trial is an ongoing phase 2 study evaluating trastuzumab combined with tucatinib [Tukysa, Seagen], a selective HER2 small-molecule inhibitor with minimal inhibition of EGFR. Early results showed an ORR of 55% among 22 evaluable patients at a median follow-up of 10.6 months.[74] This trial has subsequently expanded to enroll additional patients, as well as a separate cohort receiving tucatinib alone.[75]

The combination of trastuzumab and pertuzumab (Perjeta, Genentech), a recombinant monoclonal antibody inhibiting the heterodimerization of HER2, was evaluated in the MyPathway basket study, which included non-breast HER2-amplified tumors. In the cohort of 57 patients with treatment refractory mCRC, the ORR was 32%, with 1 patient (2%) achieving a CR.[76] The TAPUR study showed a more modest ORR of 25% in 28 patients treated with trastuzumab and pertuzumab.[77] This result may reflect the heavily pretreated nature of the population, in which 79% of the patients had received at least 3 lines of treatment.

Various antibody-drug conjugates are increasingly being used as an effective therapeutic approach in different malignancies. Trastuzumab deruxtecan (Enhertu, Daiichi-Sankyo/AstraZeneca) is composed of an anti-HER2 antibody, a cleavable tetrapeptide-based linker, and a topoisomerase I inhibitor payload. Promising results have been shown in patients with refractory HER2-positive gastric cancer in the post-trastuzumab setting. In the phase 2 DESTINY-CRC01 study, patients with HER2-expressing (IHC 1+ to 3+), *RAS* wild-type tumors were enrolled in 3 cohorts, depending on the level of HER2 expression. The ORR in cohort A (HER2+ by conventional criteria, 68% of patients) was 45%; no

patients had a confirmed response in cohorts B (IHC 2+ with negative ISH, 9% of patients) and C (IHC 1+, 23% of patients).[78] Notably, an important toxicity to consider is the risk for interstitial lung disease and pneumonitis, which was seen in 6% (5/78) of patients, with 2 fatal events; this rate appears to be lower than the rate of 13.6% seen in DESTINY-Breast01.[79] Unlike the previously described HER2-directed trials, DESTINY-CRC01 allowed previous HER2-directed treatment. DESTINY-CRC02 is an ongoing study that will treat patients who have HER2-expressing, *RAS* wild-type or *RAS*-mutant mCRC with trastuzumab at 2 different doses (5.4 and 6.4 mg/kg), while also allowing prior anti-HER2 therapy.[80]

RAS/BRAF Wild-Type Tumors: Initial Anti-EGFR Therapy and the Role of Rechallenge

As previously described, the MAPK signaling pathway has been an area of study for targeted therapy in many cancers.[81] Specifically, the anti-EGFR agents cetuximab and panitumumab (Vectibix, Amgen) target extracellular EGFR to prevent downstream effects. Anti-EGFR therapy as monotherapy can be used in third-line treatment and beyond; PFS and OS benefit was observed in patients with *KRAS* wild-type tumors in a comparison of anti-EGFR therapy with best supportive care.[82,83] Subsequent trials, including PRIME and CRYSTAL, evaluated the benefit of adding anti-EGFR therapy to doublet chemotherapy[84,85] and confirmed that the benefit is restricted to *KRAS* wild-type tumors. As the importance of tumor sidedness emerged, a reanalysis of key landmark trials, including Intergroup 80405, FIRE-3, and CRYSTAL, showed that unlike patients with right-sided tumors, those who have left-sided tumors fare better with the addition of anti-EGFR therapy.[86,87] For left-sided *RAS/BRAF* wild-type tumors, at this time, incorporating cetuximab or panitumumab into first-line systemic therapy with FOLFOX or FOLFIRI,[30] or with 5-FU, oxaliplatin, irinotecan, and leucovorin (FOLFOXIRI), is supported by data from the VOLFI study[88]; phase 3 data are awaited from TRIPLETE.[89]

Up to 50% of mCRC tumors harbor *KRAS* alterations,[90] most commonly mutations in exon 2 (codons 12 and 13). The ASCO guidelines support extended *RAS* testing in patients who have mCRC with *KRAS* and *NRAS* mutations in exons 2, 3, and 4.[91] In an exploratory analysis of the PRIME trial, additional lower-frequency *RAS* mutations outside exon 2 were predictive of inferior outcomes, and these were found in 17% of patients.[92] In addition, *KRAS* amplification is observed in fewer than 1% of patients with CRC, but it may indicate a lack of response to anti-EGFR therapy.[93] In contrast, retrospective data suggest that *EGFR* amplification may be a biomarker predictive of a favorable response.[94,95] Further prospective data are warranted to study the effect of copy number variation on response to therapy.

The increasing use of liquid biopsy to assess circulating tumor DNA (ctDNA)[96] presents an opportunity to evaluate noninvasively the clonal evolution of mCRC and acquired mechanisms of resistance to anti-EGFR therapy over time.[97,98] Using serial ctDNA samples, Siravegna and colleagues identified alterations in *KRAS*, *NRAS*, *MET*, *HER2*, *FLT3*, *EGFR*, and *MAP2K1* in patients with primary or acquired resistance to anti-EGFR therapy.[99] Salvatore and colleagues analyzed patients enrolled in the first-line PRIME and PEAK trials, who were rechallenged with anti-EGFR therapy at the third line of treatment or later. Median OS was 14.2 months after rechallenged,[100] which was favorable vs the historical median OS of 6 to 7 months in the refractory setting, with the use of TAS-102 and regorafenib (Stivarga, Bayer).[101,102] Parseghian and colleagues demonstrated that *RAS* and *EGFR* clones decay exponentially, with a half-life of 4.4 months validated in an external cohort.[103] In addition, the ORR among patients who were rechallenged for more than 2 half-lives after prior anti-EGFR therapy was numerically highest (32% vs 20% for 1-2 half-lives vs 16% for <1 half-life), but this difference was not statistically significant. Other anti-EGFR rechallenge trials that have incorporated ctDNA have shown ORRs ranging from 16% to 21%,[104,105] but time from previous anti-EGFR therapy is variable. The results of ongoing trials of anti-EGFR rechallenge guided by ctDNA results are pending.[106-109]

KRAS G12C–Mutated Tumors

Up to 3% to 4% of metastatic CRC cases harbor a *KRAS* G12C mutation, which is an oncogenic driver that predicts resistance to anti-EGFR therapy.[110] Sotorasib (Lumakras, Amgen) is an irreversible small-molecule inhibitor of *KRAS* G12C that binds to the inactive GDP-bound state.[111] In the phase 1 trial by Hong and colleagues, which included multiple tumor histologies, 42 of 129 enrolled patients had mCRC. Among these, sotorasib treatment was associated with an ORR of 12%.[112] Adagrasib is another small-molecule inhibitor of *KRAS* G12C, and monotherapy was associated with an ORR of 22% in the KRYSTAL-1 trial[113]; when used in combination with cetuximab, the ORR was 39%. The ongoing KRYSTAL-10 study is comparing combination adagrasib and cetuximab in the second-line setting vs doublet chemotherapy with or without anti-VEGF therapy and will provide phase 3 efficacy data, as well as guidance on how to sequence *KRAS* G12C inhibitors in the treatment of mCRC.[114]

NTRK Fusions

The tropomyosin receptor kinase (TRK) family comprises the transmembrane proteins TRKA, TRKB, and TRKC, which are found in neuronal tissue.[115] Translocations in neurotrophic tyrosine receptor kinase (*NTRK*) genes are enriched in relatively rare tumor histologies[116,117] and are observed in fewer than 1% of mCRC cases.[118] *NTRK* fusions are more likely to be found in MSI-H/dMMR tumors than in MSS/pMMR tumors, with frequencies of 5% vs 0.4%, respectively. The frequency increases to 15% in tumors that are MSI-H/dMMR and also *RAS/BRAF* wild-type.[118] Fusion of one of the *NTRK* genes leads to the production of an oncoprotein, with constitutive TRK activation.[117] At this time, no clear prognostic association has been observed, as seen in a cohort of *NTRK* fusion–positive cancers,[119,120] but TRK fusion proteins are clinically actionable with the availability of the TRK inhibitors larotrectinib (Vitrakvi, Loxo) and entrectinib (Rozlytrek, Genentech), which are approved in the United States in a tumor-agnostic fashion.

Larotrectinib is an inhibitor of TRKA, TRKB, and TRKC, and a pooled analysis of 3 trials that included both pediatric and adult patients with various tumor histologies showed an ORR of 79% and a CR rate of 16%.[120] Median PFS was 28.3 months, representing a very durable strategy. Of the 153 patients, 8 had CRC, and 4 (50%) of these patients demonstrated a response.

Entrectinib has multiple targets and inhibits TRKA, TRKB, and TRKC in addition to c-*ROS* oncogene 1 (*ROS1*) and anaplastic lymphoma kinase (ALK). It was studied in the STARTRK-1, STARTRK-2, and ALKA-372-001 trials, which enrolled adult patients only. In a pooled analysis of 54 patients, the ORR was 57% and the CR rate was 7%; median PFS was 11.2 months.[121] Of these patients, 4 (7%) had CRC, and 1 patient (25%) responded. Entrectinib has not been compared head-to-head with larotrectinib, and resistance mechanisms are currently being investigated. In one patient who demonstrated resistance to entrectinib, 2 mutations were seen in the *NTRK1* kinase domain on ctDNA testing (Gly595Arg and Gly667Cys). Future studies are warranted to confirm that these mutations lead to secondary resistance to entrectinib in a wider population.

Selitrectinib (also known as LOXO-195) and repotrectinib are next-generation TRK inhibitors[122,123] that are the subject of ongoing studies to see if they are effective in the next-line setting.[124,125] Because TRK fusions are less commonly found in CRC and data are limited, it is unclear at this time whether NTRK inhibitors are less effective in CRC than in other tumor groups owing to the numerically lower ORR. Sequencing data are not available but are recommended in the NCCN guidelines in a non–first-line setting.[30]

Guidelines for Biomarker Testing

In the mCRC setting, the NCCN guidelines support testing for *RAS* (*KRAS* and *NRAS* exons 2, 3, and 4) and *BRAF* mutations, which can be tested individually or through an NGS panel. MMR or MSI testing should be performed universally for all patients with newly diagnosed CRC to identify possible cases of HNPCC and to plan treatment. HER2 testing should also be performed for patients with *KRAS/BRAF* wild-type tumors, to plan for therapeutic options in the post first-line setting or to consider enrollment in clinical trials, although some are now allowing accrual of *KRAS*-mutant cases. With the increasing accessibility of NGS panels, clinicians are obtaining more information on novel predictive biomarkers, and precision medicine can be pursued. However, the number of patients who will benefit from some of these targeted strategies still remains numerically low, and this will have to be balanced against the financial cost of NGS panels.[126] Liquid biopsy for ctDNA is another modality that is increasingly available to help detect minimal residual disease, monitor response to therapy, and track clonal evolution over time, although its use remains largely within the research realm.[96] The use of liquid biopsy for ctDNA is not integrated into current guidelines, but that may change in the near future as more data come out and policies in coverage evolve.[127]

Conclusion

Although traditional chemotherapy remains a backbone of mCRC treatment, significant advances in personalizing therapy have been made by using a biomarker-driven approach based on molecular profiling. As more knowledge is gained about the genomic landscape of mCRC and its evolution through sequential treatments, future studies will be needed to develop strategies against currently undruggable molecular alterations and to overcome resistance mechanisms.

Disclosures

The authors have no disclosures to report.

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