Targeted Therapy and the Use of Molecular Profiling in Metastatic Colorectal Cancer

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Abstract

Metastatic colorectal cancer (mCRC) is the third leading cause of cancer-related mortality in the United States, but survival rates for advanced CRC have improved significantly in the past 15 years. This improved survival is due, in large part, to more effective chemotherapy, but improvements have also been attributed to the incorporation of therapies that either target, or are guided by, the multiple aberrant signaling pathways involved in the growth and spread of colorectal cancer cells, including the VEGF, EGFR, RAS/RAF, and HER2 signaling pathways, as well as genetic changes induced by mismatch repair enzyme deficits and the resultant microsatellite instability. Targeted treatments directed toward inhibiting these pathways have improved survival rates beyond those achieved with standard chemotherapy. This review provides an update on targeted agents used in mCRC and the impact that specific, defined predictive biomarkers have on patient selection and, ultimately, patient outcome.

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Introduction

Colorectal cancer (CRC) is the third leading cause of cancer-related death in the United States, affecting men and women equally.[1] In 2017, there will be an estimated 135,430 new cases, with 50,260 deaths due to CRC.[1] Approximately 20% of patients are diagnosed with advanced or metastatic disease on presentation, and 50% of all CRC patients will develop progressive disease and metastases over time. The prognosis for patients with advanced disease without treatment is poor, with a median overall survival of 6 months. However, advances in systemic therapy with combination chemotherapy using a fluoropyrimidine, irinotecan, and oxaliplatin have improved survival rates up to 20 months.[2]

The development of targeted agents aimed at blocking key pathways involved in CRC cell growth and invasion further improved survival through the latter part of the first decade of the 2000s. The VEGF pathway inhibitors—primarily bevacizumab, but more recently ziv-aflibercept and ramucirumab—increased survival rates, compared with chemotherapy alone.[3-9]

However, any predictive marker for selecting patients who would benefit most from VEGF pathway inhibitors has been elusive, and will not be discussed herein.

By contrast, other therapies, including those either targeting, or guided by, molecular abnormalities in the EGFR, RAS/RAF, and HER2 pathways, as well as immunotherapy for tumors with high levels of microsatellite instability, have defined predictive biomarkers, and they have demonstrated significant impact in well-selected patients.[10-13]

This review will focus on molecularly targeted agents in metastatic colorectal cancer (mCRC) that have defined predictive biomarkers. We will also comment on the role of "molecular profiling" in identifying these subpopulations of patients who will benefit from appropriately targeted therapy, and the magnitude of benefit of those therapies.

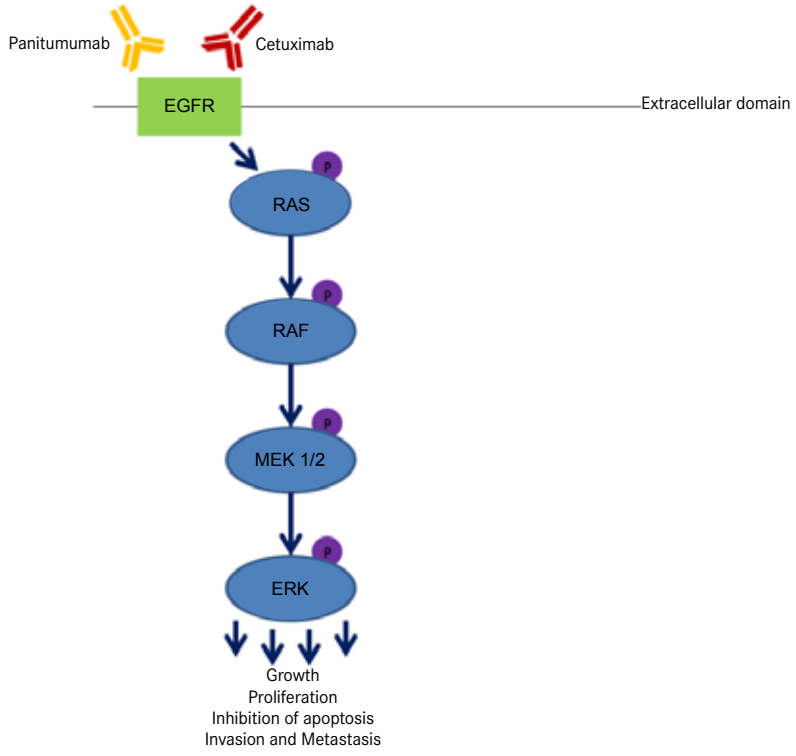
Targeting the *EGFR*

The *EGFR* is overexpressed in approximately 60% to 80% of CRCs.[14] Activation of the EGFR stimulates downstream signaling through the RAS, RAF, MAPK, and ERK pathways, leading to activation of several pathways involved in cell survival, proliferation, and the ability of cancer cells to metastasize.[15,16,17]

Two anti-EGFR treatments have been approved for patients with mCRC: cetuximab and panitumumab.[14,18] Both drugs are monoclonal antibodies that target the *EGFR*, preventing receptor activation and thereby inhibiting the signaling via the RAS/RAF/MAPK/ERK pathway (**Figure**). Both were first approved in the refractory disease setting with *EGFR* as the sole predictive biomarker of response.

Cetuximab was initially studied by Cunningham and colleagues.[14] In the BOND trial, 329 chemotherapy-refractory patients with CRC were randomized to receive cetuximab and irinotecan versus cetuximab alone. To be eligible, either the primary tumor or a metastatic lesion must have expressed *EGFR* by immunohistochemistry (IHC).[14] The objective response rate (ORR) was 22.9% (95% CI, 17.5%-29.1%) in the cetuximab-plus-chemotherapy arm and 10.8% (95% CI, 5.7%-18.1%) in the cetuximab-alone arm ($P = .007$).[14] The progression-free survival (PFS) improved to 4.1 months in the combination arm, compared with 1.5 months with single-agent cetuximab.[14] The overall survival (OS) rate did not improve when compared with cetuximab alone in EGFR-expressing patients who had progressed through irinotecan-based therapy.[14] Of note, the degree of *EGFR* expression did not correlate with response, but patients with skin reactions after treatment with cetuximab had higher response rates than those without skin reactions.[14] Grade 3 or 4 adverse

FIGURE. The RAS-RAF Pathway



EGFR indicates epidermal growth factor receptor; P, phosphorylation.

events (AEs) most commonly included diarrhea (21% in the combination arm vs 2% in the monotherapy arm) and neutropenia (9.4% in the combination arm vs 0% in the monotherapy arm).[14]

Panitumumab was shown to improve outcomes when compared with best supportive care (BSC) in the trial by Van Cutsem and colleagues.[18] Randomization of 463 chemotherapy-refractory patients to single-agent panitumumab improved ORR and PFS but not OS (hazard ratio [HR], 1.00; 95% CI, 0.82%-1.22%; *P* = .81) when compared with BSC alone. The lack of OS benefit was thought to be due to the confounding variable of the crossover design of the study.[18]

The Impact of pan-*RAS* Testing

The above-mentioned trials, however, were done in the "pre-*RAS*" testing era.[14,18,19] Posthoc analysis of these trials, as well as of several additional pivotal trials with cetuximab and panitumumab, have shown the benefit of *KRAS* testing, and more recently "pan-*RAS*" testing, on outcome in patients with mCRC (**Table 1**).

RAS and its subtypes, *KRAS* and *NRAS* (and likely *HRAS*), as well as the downstream signaling effector RAF, have been important markers in the treatment of CRC.[20] When genetic mutations occur that result in constitutive activation of the RAS or RAF enzymes, signaling is activated down the RAS/RAF/MAPK/ERK pathway irrespective of inhibition of the *EGFR*, upstream of the active enzyme. Thus, logically, treatment with cetuximab or panitumumab on tumors with *RAS* or *RAF* gene mutations has generally demonstrated no benefit. This is true for other less-frequent *RAS* mutations, and may be the case for *BRAF*, but this has not been well established. *KRAS* mutations are present in approximately 40% of all CRC patients and can be seen in both early- and late-stage disease.[21,22] The most common activating mutations occur in codon 12 and 13 of exon 2 of the KRAS protein. Within codon 12, the G12D and G12V mutations are the most common, occurring 13% and 9% of the time, respectively. In codon 13, G13D is the most frequent mutation, occurring in 8% of *KRAS*-mutated CRC. The frequencies of *NRAS* and *RAF* mutations are less common; they are seen in approximately 2% and 9% of patients, respectively.[22] Altogether, "pan-RAS" wild-type (WT) tumors—those with WT *KRAS*, *NRAS*, likely *HRAS*, and *RAF* genes—make up only about 40% of CRCs, but there is a significant chance of benefit with anti-EGFR therapies in pan-*RAS* WT tumors.[23]

A number of studies have looked at mutations in the RAS pathway and their predictive and prognostic significance in colon cancer. When the initial anti-EGFR therapy trials were re-evaluated, taking into consideration pan-RAS status, it was clear that the magnitude of benefit of anti-EGFR therapy was much greater when restricted to patients with pan-RAS WT tumors only.

In a study by Jonker and colleagues (the joint Canadian/Australasian CO.17 trial) cetuximab was compared with BSC in *EGFR*-expressing mCRC and showed improved survival (HR for death, 0.77; 95% CI, 0.64-0.92; *P* = .005) in addition to improved PFS (HR, 0.68; 95% CI, 0.57-0.80, *P* <.001) and ORR.[19] *KRAS* mutational status was not initially evaluated, but a posthoc analysis of the trial revealed that tumors with *KRAS* exon 2 mutations treated with cetuximab had a worse outcome compared with those without the mutation or with WT *KRAS* status, with an OS of 9.5 months for the patients with *KRAS* WT tumors versus 4.8 months for the patients with *KRAS*-mutated tumors (HR, 0.55; 95% CI, 0.41-0.74, *P* <.001).[23]

When *KRAS* mutational status was examined in the posthoc analysis of the Van Cutsem study of panitumumab versus BSC, PFS was significantly greater in the WT *KRAS* group (12.3 weeks; HR, 0.45; 95% CI, 0.34-0.59) compared with the mutated *KRAS* group (7.3 weeks; HR, 0.99; 95% CI, 0.73-1.36).[24] The nonmutated *KRAS* group also had an improved OS compared with the mutated arm.[18]

Given the predictive value of identifying *RAS* mutations in mCRC, the concept of extended *RAS* analysis was first initiated by the PRIME and

TABLE 1. ***BRAF*** **and** ***EGFR*** **inhibition**

Cetuximab										
First-line										
		KRAS WT/mut			RR *KRAS* WT		PFS *KRAS* WT		OS *KRAS* WT	
Study or author	*KRAS* analysis	N	%	Treatment	%	OR;*P*	Months	HR;*P*	Months	HR;*P*
OPUS[70]	113 120	134/99	58/42	FOLFOX+C vs FOLFOX	61 37	OR, 2.544; *P* = .11	7.7 7.2	HR, 0.57; *P* = .163	NA	NA
Bokemeyer[68]	156 159	179/136	57/43	FOLFOX vs FOLFOX+C	57 34	OR, 2.551; *P* = .0027	8.3 7.2	HR, 0.567; *P* = .0064	22.8 18.5	HR, 0.855; *P* = .39
Ye[75]	All	All *KRAS* WT		FOLFIRI/- OX+C vs FOLFIRI/-OX	57.1 29.4	*P* <.01	10.2 5.8	HR, 0.6; *P* = .004	30.9 21	HR, 0.54; *P* = .13
Crystal[70]	277 263	348/192	64/36	FOLFIRI+C vs FOLFIRI	59.3 43.2	OR, 2.069; *P* <.001	9.9 8.7	HR, 0.68; *P* = .02	24.9 21	HR, 0.84; *P* = .44
Van Cutsem[34]	533 531	667/397	63/37	FOLFIRI vs FOLFIRI+C	57.3 39.7	NA	9.9 8.4	HR, 0.696; *P* = .0012	23.5 20	HR, 0.796; *P* = .0093
MRC COIN[67]	648 668	729/565	55/43	FOL-/CAPOX+C vs FOL-/CAPOX	64 57	*P* = .049	8.6 8.6	HR, 0.96; *P* = .6	17 17.9 *RAS/BRAF* WT 19.9 20.1	HR, 1.04; *P* = .67 HR, 1.02; *P* = .86
NORDIC-VII[72]	155 169 174	303/195	61/39	FLOX+C FLOX(inter)+C FLOX	47 46 51	OR, 0.96; *P* = .89	7.9 8.3 7.3	HR, 1.07; *P* = .66	22 20.1 20.6	HR, 1.14; *P* = .48 *P* = .66
New EPOC[69]	129 128	NA	NA	FOLFOX/CAPOX/FOLFIRI+C vs FOLFOX/CAPOX/FOLFIRI	70 62	*P* = .59	14.1 20.5	HR, 1.48; *P* = .03	39.1 Not reached	HR 1.49 *P* = .16
Beyond first-line										
Karapetis[23]	198 196	230/164	58/42	BSC+C vs BSC	12.8 0		3.7 1.9	HR, 0.40; *P* <.001	9.5 4.8	HR, 0.55; *P* <.001
Panitumumab										
First-line										
PRIME[65]	546 550	656/440	60/40	FOLFOX+P vs FOLFOX	55 48	OR, 1.35; *P* = .68	9.6 8	HR, 0.80; *P* = .02	23.9 19.7	HR, 0.83; *P* = .72
Douillard[26]	*RAS* WT/any *RAS* mut	512/548	48/52	FOLFOX+P vs FOLFOX			10.1 7.9	HR, 0.72; *P* = .004	26 20.2	HR, 0.78; *P* = .43
Douillard[70]	590	656/440	60/40	FOLFOX+P vs FOLFOX	57 48	OR, 1.47; *P* = .02	10 8.6	HR, 0.80; *P* = .01	23.9 19.7	HR, 0.83; *P* = .03
Second-line and beyond										
Amado[10]	208 219	243/184	57/43	BSC+P vs BSC	17 3		3 1.8	HR, 0.45; *P* <.001	8.1 7.6	HR, 0.67; both arms combined
Douillard[66]	541 542	597/486	55/45	FOLFIRI+P vs FOLFIRI	35 10	*P* <.1	5.9 3.9	HR, 0.73; *P* = .004	14.5 12.5	HR, 0.85; *P* = .12
Peeters[71]	NA	NA	NA	FOLFIRI+P vs FOLFIRI	36 10	OR, 5.5; *P* <.0001	6.7 4.9	HR, 0.82; *P* = .023	14.5 12.5	HR, 0.92; *P* = .37
PICCOLO[72]	230 230	460	100	IRI+P vs IRI	34 12	*P* <.0001		HR, 0.78; *P* = .015	10.4 10.9	HR, 1.01; *P* = .91

BSC indicates best supportive care; C, cetuximab; CAPOX, capecitabine and oxaliplatin; FLOX, bolus 5-fluorouracil with leucovorin and oxaliplatin; FOLFIRI, 5-fluorouracil with leucovorin and irinotecan; FOLFOX, 5-fluorouracil with leucovorin and oxaliplatin; HR, hazard radio; IRI, irinotecan; mut, mutant; NA, not available; OR, odds ratio; ORR, overall response rate; OS, overall survival; P, panitumumab; PFS, progression-free survival; RR, response rate; WT, wild-type.

PEAK studies.[25] In the PRIME study, 512 patients with mCRC who were treated with FOLFOX4 (folinic acid, fluorouracil, oxaliplatin) with or without panitumumab were assessed according to *RAS* (*KRAS* or *NRAS*) or *BRAF* status.[26] Patients who were WT for extended *RAS* analysis including *KRAS* and *NRAS* exon 2, 3, 4 had a 5.8-month OS benefit with the addition of anti-EGFR therapy compared with chemotherapy alone (26.0 vs 20.2; *P* = .04).26 The PEAK study looked at extended *RAS* analysis including exon 2, 3, 4 of *KRAS* and *NRAS* in patients with WT *KRAS* mCRC.[27] It compared FOLFOX6 plus bevacizumab versus FOLFOX6 plus panitumumab in 278 patients with KRAS WT exon 2 mCRC. Like the PRIME study, the PEAK trial showed an improved PFS and OS in WT *RAS* compared with *KRAS* exon 2 mutated CRC for patients treated with panitumumab.[27] In the *RAS* WT patients, improved PFS rates were seen with panitumumab (HR, 0.65; 95% CI, 0.44-0.96; *P* = .029). OS was 41.3 months in the panitumumab arm versus 28.9 months in the bevacizumab arm (HR, 0.63; 95% CI, 0.39-1.02; *P* = .58).[27] The results

of these 2 studies suggest that mutations in the RAS pathway, including those beyond *KRAS* exon 2 mutations, are predictive of a lack of response to anti-EGFR therapy for patients with mCRC.

Some data from Tejpar and colleagues suggest that patients with the *KRAS* G13D mutation may derive benefit when treated with cetuximab in combination with chemotherapy, compared with other *KRAS* mutations, but the effectiveness is still less than that seen in *KRAS* WT patients.[28] Although this study highlights the variations in tumor biology seen in *KRAS*-mutated CRC, more clinical data are needed.

Interestingly, not all *KRAS* WT CRC responds to anti-EGFR treatment either, suggesting additional mutations also confer resistance.[16,22] Emerging data indicate that the location of the primary tumor in mCRC has a role in predicting a response to EGFR inhibitors. Patients with left-sided *KRAS* WT tumors, located between the splenic flexure and rectum, were shown to have improved OS if first-line treatment included cetuximab compared with bevacizumab (37.5 vs 16.4 months; HR, 1.97; 95% CI, 1.56-2.48).[29] A number of additional genes are known to be somatically mutated and have been studied in response to anti-EGFR therapy.[30] A study by Peeters and colleagues used next-generation sequencing on mCRC tissue and found additional mutations in *NRAS, BRAF, PIK3CA, PTEN, TP53, EGFR, AKT1*, and *CTNNB1*.[30] Patients with WT *KRAS* but mutated *NRAS* or *BRAF* did not respond to panitumumab; however, if patients were WT for *KRAS, NRAS*, and *BRAF*, the ORR was 18%.[30]

Fifteen years of clinical trials of anti-EGFR therapies, and more recent incorporation of *RAS/RAF* testing, have demonstrated that patients with pan-*RAS* WT tumors derive significant benefit from anti-EGFR therapy, while patients with *RAS/RAF*-mutated tumors derive little to no benefit. In fact, some studies have shown a detrimental effect and decreased OS (rather than just a lack of benefit) in patients with *KRAS*-mutated CRC who are treated with an EGFR inhibitor.[10,12] Therefore, pan-*RAS* testing to evaluate mutations in *KRAS, NRAS*, and *BRAF* is an accepted standard-of-care practice in patients with mCRC. With 60% of tumors being *RAS/RAF*-mutated, the challenge in the coming years will be to identify novel therapies that target *RAS/RAF*-mutated tumors specifically.

BRAF Mutations

BRAF is a subset of the *RAS* family of oncogenes, which is mutated in approximately 10% of CRC cases[31,32] and has been associated with decreased survival.[10,33] The most common *BRAF* mutation is located in exon 15, resulting in a substitution from valine to glutamic acid at position 600 within the *BRAF* kinase domain (V600E). This leads to constitutive activation of the MAPK signaling pathway. Standard chemotherapy in combination with EGFR inhibitors in patients with mCRC who harbor the *BRAF* V600E mutation is less effective than in those with *BRAF* WT tumors.[34] In patients with *KRAS* WT/*BRAF*-mutated tumors who were treated with FOLFIRI (5-fluorouracil with leucovorin and irinotecan) plus cetuximab, there was no statistically significant improvement in OS with the addition of anti-EGFR therapy.[34] The lack of response is also seen with anti-EGFR inhibitors that are given without concurrent *BRAF* inhibition.[35] In a retrospective analysis, patients with mCRC whose tumors were *BRAF* V600E–mutated were resistant to treatment with cetuximab or panitumumab, which was also confirmed in a cell-line model using colorectal tumor cells expressing the mutated *BRAF* V600E allele.[35] However, when these cells were treated with a combination of cetuximab and sorafenib (an approved small molecule kinase inhibitor targeting *BRAF*), there was a synergistic effect causing cell death.[35] Unfortunately, vemurafenib, another oral *BRAF* V600E inhibitor, showed disappointing results when used as a single agent in *BRAF*-mutated mCRC in the refractory setting.[35] One patient had a confirmed partial response (PR) out of 21 patients who were treated.[36] This is in stark contrast to the response rates of 60% to 80% seen in vemurafenib-treated patients with melanoma who harbor the identical *BRAF* V600E mutation.[37] This resistance is thought to be due to inadequate suppression of the MAPK pathway by *BRAF* inhibition alone, due to an incomplete ERK suppression (located downstream of BRAF).[37]

There was initial optimism for combining the *BRAF* inhibitor dabrafenib with trametinib, a MEK inhibitor that targets downstream of *BRAF* and MAPK, given that this combination has been effective in *BRAF* V600E–mutated melanoma. Forty-three patients with *BRAF* V600E–mutated mCRC were treated, and 5 patients (12%) achieved a PR, including 1 patient with a durable complete response (CR) extending over 36 months.[38] The median PFS was 3.5 months, compared with 2.5 months seen with standard chemotherapy. Nine patients had biopsies during treatment, which revealed decreased levels of phosphorylated ERK, compared with pretreatment biopsies. However, there was not a more robust efficacy despite dual inhibition of *BRAF* and mitogen-activated protein kinase kinase (MEK).[39]

More recent trials combining BRAF and EGFR inhibition have shown promising results (**Table 2**). When vemurafenib was combined with cetuximab and irinotecan, early-phase data demonstrated a promising PFS of 7.7 months in previously treated patients with *BRAF* V600E–mutated, *KRAS* WT tumors.[12] There was a recent update of this initial trial at the 2017 Gastrointestinal Cancers Symposium (GI ASCO) conference by Kopetz and colleagues. One hundred and six patients with *BRAF* V600E–mutated extended *RAS* WT mCRC were randomized to irinotecan and cetuximab with or without vemurafenib.[40] PFS in the vemurafenib arm was 4.4 months versus 2 months in the irinotecan and cetuximab–only arm, with response rates of 16% versus 4%, respectively.[40] Updated analysis presented at GI

TABLE 2. BRAF and EGFR Inhibition

Study (citation)	Treatment	ORR	PFS (months)	OS
Kopetz[40]	Cetuximab + irinotecan Cetuximab + irinotecan + vemurafenib	4% 16%	2 4.4	5.9 9.6
Corcoran[41]	Panitumumab + dabrafenib + trametinib	18% PR 67% SD	Not reached	Pending

ORR indicates overall response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease.

ASCO 2017 revealed a median OS of 9.6 months in the vemurafenib arm versus 5.9 months in the irinotecan and cetuximab–only arm (HR, 0.73; 95% CI, 0.45-1.17; *P* = .19). The lack of survival benefit is thought to be due to crossover.

Another study evaluated the efficacy of combining panitumumab with dabrafenib and trametinib in *BRAF* V600E–mutated mCRC.[41] Two of the 120 treated patients had concomitant *BRAF* V600E and *RAS* mutations at baseline. The combination of all 3 drugs achieved an 18% PR or better, with 67% of patients achieving stable disease. Comparatively, the PR/CR rate in the dabrafenib-and-panitumumab arm was 10%, but was 0% for the trametinib-and-panitumumab arm. Stable disease was seen in 80% and 53%, respectively. Median PFS for the triple combination had not been reached at the study end date. Of 12 patients with PR/CR or stable disease, 58% had a detectable *RAS* mutation on progression of disease. Updated analysis is pending.

It is important to note that *BRAF* mutations in mCRC confer a poor prognosis independent of the predictive value and possible efficacy of the combination with EGFR and MEK inhibitors, as discussed above.[10,33] This worse prognosis will need to be considered as definitive trials are developed.

MMR-Deficient CRC and Immunotherapy

Tumors that have defects in the mismatch repair (MMR) system accumulate hundreds to thousands of somatic mutations in the microsatellite regions of DNA that are normally repaired.[42,43] A defect in MMR (also called MMR deficient) is a surrogate for microsatellite instability (MSI), and MSI is further subdivided into MSI-high (MSI-H) and MSI-low (MSI-L). Tumors with an intact mismatch repair system (MMR proficient) are considered microsatellite stable (MSS). Dysregulation of the MMR system is caused primarily by mutations in the *MLH1*, *MSH2*, *MSH6*, and *PMS2* genes (though other genes can be implicated as well).[43,44] Hereditary forms of MMR deficiency can occur, which is known as hereditary nonpolyposis colorectal cancer or Lynch syndrome.[45] This disorder is observed in 10% to 15% of sporadic cases of colon cancer; it is most commonly caused by a hypermethylation mutation in the *MLH1* gene.[45]

Approximately 10% to 15% of sporadic GI cancers also carry the MSI-H phenotype.[46,47] MSI-H is present in 15% of early-stage CRC.[41] MSI-H is rare in metastatic disease, with incidence rates of about 4%, and the prognosis is unclear. MSI-H tumors typically lack mutations in *TP53, KRAS,* and *APC,* which are commonly mutated genes seen in MMR-proficient CRC.[47,48] While MSI status is used as a prognostic marker in early-stage CRC, its role as a predictive marker for chemotherapy is conflicting. Typically, MMR-deficient (MSI-H) tumors are less aggressive than MMR-proficient (MSI-L, or MSS) tumors, with a better overall prognosis.[47] Numerous studies have shown that patients with MSI-H tumors have better survival rates in early-stage disease. In a meta-analysis pooling 32 eligible studies including 1277 MSI samples, MMR-deficient (MSI-H) tumors were associated with a 35% reduction in the risk of death compared with those that were MMR-proficient (MSS).[49] However, a study by Goldstein and colleagues showed that MSI-H mCRC did not have the improved outcome that was observed in early-stage CRC.[48] Additionally, the *BRAF* V600E mutation is a poor prognostic factor that is seen in MSI-H mCRC.[48] *BRAF* mutations are only seen in MSI-H sporadic CRC, and they can be used to differentiate between sporadic and hereditary forms of MSI-H CRC.[48]

Clinically, MMR-deficient (MSI-H) CRC has been shown to possess a highly activated lymphocyte microenvironment.[43,50] MMR-deficient (MSI-H) tumors are also known to have an increased stromal inflammatory reaction.[45] These tumors carry a higher number of cytotoxic lymphocytes that infiltrate the tumor architecture itself.[45] These lymphocytes are seen in close proximity to tumor cells undergoing apoptotic death.[45] The increased cytotoxic immune response against tumor cells is thought to be related to the increased mutational load in MMR-deficient (MSI-H) tumors, allowing for greater immunogenicity.[45] The accumulation of irregular proteins provides a source of abnormal peptides to be presented to T lymphocytes.[47] These cytotoxic T lymphocytes are also known to overexpress immune checkpoint–related proteins in the microenvironment, including PD-1, PD-L1, CTLA-4, lymphocyte-activation gene 3, and indoleamine-pyrrole 2,3-dioxygenase.[50] The amount of lymphocyte infiltration into the tumor is an important predictor of relapse and OS.[50]

Cancer cells have an innate ability to maintain an immunosuppressive microenvironment, thus escaping the immune system mechanisms that target foreign cells for destruction.[44] PD-L1 on tumor cells binds PD-1, which is expressed on the cell surface of T lymphocytes, thereby inhibiting the activation of PD-1 and evading tumor-cell killing.[44] The expression of PD-L1 on the surface of tumor cells is a predictive marker that is used to predict response to PD-1 blockade.[42]

Preclinical data suggested that continuous antigen exposure to cytotoxic T lymphocytes may induce an exhausted or less vigorous state of activity in which T-cell effectiveness and transition to memory T cells are impaired.[47] Inhibiting the PD-1 pathway with novel agents may restore T-lymphocyte function, resulting in tumor-cell death by the immune system.[13] The immune infiltration of cytotoxic lymphocytes is suggested to be a better predictor of survival than the current IHC methods used to stage colon cancer.[51]

Initial studies with PD-1 blockade in CRC were limited but promising.[52] One of 33 patients treated with the humanized monoclonal immunoglobulin G4 (IgG4) anti–PD-1 antibody nivolumab had MSI-H mCRC. The patient had progressed through multiple lines of treatment and eventually was treated with single-agent nivolumab. The patient achieved a complete remission and showed no evidence of disease recurrence 3 years out from treatment. PD-L1 expression was seen in his original tumor tissue with evidence of infiltrating cytotoxic T cells.[13]

Pembrolizumab is a humanized monoclonal IgG4 kappa isotype anti–PD-1 antibody that was tested in a phase II study in patients selected specifically for their MSI-H mCRC status.[53] When compared with patients with MSS tumors, MSI-H patients had an improved ORR (40% vs 0%) and PFS (78% vs 11%) at 20 weeks.[53] Whole-exome gene sequencing also revealed that a high somatic mutational load was associated with improved PFS. This included patients with inherited and sporadic forms of MSI-H tumors.[53]

A similar study was more recently published in abstract form by Overman and colleagues.[54] Nivolumab was tested in patients with mCRC

with and without ipilimumab, a humanized anti–CTLA-4 monoclonal antibody.[54] In patients with MSI-H tumors, initial results with nivolumab showed a PFS of 5.3 months and a median OS of 16.3 months. The combination arm had not reached either the PFS or OS endpoints. A pooled PFS of 1.4 months was seen in the non–MSI-H tumors.[54] AEs included GI toxicity and fatigue.[54] A recent update of the nivolumab monotherapy arm revealed an ORR of 31% with a 69% disease control rate. An updated PFS at 12 months was 48.4%. The duration of response and OS have not been reached. These responses are irrespective of PD-L1 expression or *KRAS* and *BRAF* mutation status.[55]

The identification of MMR-deficient (MSI-H) CRC defines a subset of tumors that have specific molecular, pathologic, and clinical features that have shown to improve survival,[56] and this justifies routine testing for MMR status in all patients with mCRC. The National Comprehensive Cancer Network guidelines recommend that all mCRCs be evaluated for MSI status, and both drugs, pembrolizumab and nivolumab, are approved treatment options. [51,53]

CRC and HER2-Targeted Treatment

HER2 overexpression, which has a prevalence of 5% in CRC, has been identified as a novel potentially actionable molecular target. Previous trials that added HER2-targeted therapy to chemotherapy were inconclusive.[56-60] One study evaluated the combination of 5-fluorouracil, oxaliplatin, and trastuzumab in patients with mCRC who had progressed on treatment containing 5-flurouracil and/or irinotecan.[59] It closed early due to insufficient accrual. Another study combined trastuzumab with irinotecan in *HER2*-overexpressing CRC.[60] Nine patients out of 138 screened had tumors with *HER2* overexpression. These 9 patients were enrolled into the study and only 7 were counted for data collection. Partial responses were seen in 5 of 7 patients.[60] This study also closed early due to low accrual.[60] Monotherapy with HER2-targeted treatment with a tyrosine-kinase inhibitor (lapatinib) or monoclonal antibody (trastuzumab) was also initially ineffective in early preclinical studies; however, the combination of the 2 showed sustained tumor control.[57] The success of combination HER2-targeted therapy is thought to be related to the association of dual EGFR/HER2 inhibition by lapatinib and trastuzumab targeting the HER2 heterodimer.[58]

Because the combination of trastuzumab and lapatinib has been used as a standard treatment option in *HER2*-positive breast cancer,[61] Sartore-Bianchi and colleagues used trastuzumab and lapatinib in combination in patients who were *KRAS* exon 2 WT and *HER2*-positive in the HERACLES study. They defined HER2 positivity as either a 3+ score in more than 50% of cells by IHC, or 2+ and having a HER2:CEP17 (chromosome enumeration probe 17) ratio >2 in more than 50% of cells by fluorescence in situ hybridization.[55] A total of 914 patients were screened, with 5% being identified as *KRAS* WT and *HER2*-amplified.[55] Twenty-seven patients were eligible to enroll in the trial. These patients were heavily pretreated and had progressed through all prior standard chemotherapy including 5-fluorouracil, irinotecan, oxaliplatin, and anti-VEGF and anti-EGFR antibodies.[55] Nevertheless, in this heavily pretreated population, the combination of trastuzumab and lapatinib resulted in a 30% ORR according to Response Evaluation Criteria in Solid Tumors v1.1 criteria, with durable responses, and a median duration of 38 weeks.[55] *HER2* is also suggested to be an early molecular alteration that persists during tumor progression, as Sartore-Bianchi and colleagues saw that HER2 was matched between the primary tumor and metastatic lesions. A follow-up study (HERACLES-RESCUE) is accruing to evaluate ado-trastuzumab emtamsine (T-DM1) in patients who have progressed on trastuzumab and lapatinib.[62] T-DM1 is an antibody–drug conjugate that binds HER2-expressing cells; the conjugate releases emtamsine within the cell, resulting in cytotoxicity.

Hurwitz and colleagues have recently presented data from the MyPathway study, evaluating the combination of trastuzumab with pertuzumab in HER2-amplified or HER2-overexpressed mCRC.[63] Pertuzumab is a monoclonal antibody that targets the HER2 dimerization domain. Inhibiting dimerization blocks downstream signaling, which inhibits cell growth and causes apoptosis. The 34 patients enrolled in the study received standard doses of trastuzumab and pertuzumab until disease progression or unacceptable toxicity. The ORR was similar to the HERACLES trial at 37.5%, with a median duration of response of 11.1 months.

Interestingly, amplification of the *HER2* gene does not seem to be related to mutations in *KRAS*, *NRAS*, or *BRAF*, but it has been shown to confer some resistance to anti-EGFR therapy.[57,58] Two recent studies showed that HER2 amplification allows for downstream signaling activation, even when EGFR inhibition has resulted in drug resistance.[56,57] HER2 can therefore be considered a negative biomarker of anti-EGFR resistance but a positive marker of anti-HER2 targeted agents.[58]

Conclusion: The Need for Broad Molecular Testing in All Patients With mCRC

Molecular profiling is an important tool in selecting the right patient for specific targeted agents. Pan-*RAS* testing that evaluates for *KRAS, NRAS*, and *BRAF* mutations is important to determine which patients are likely to derive benefit from EGFR inhibitors like cetuximab or panitumumab, and this testing is nationally recognized for mCRC prior to initiation of therapy. Only patients with WT *RAS* mCRC have seen significant improvement in PFS and OS, while treating mutated-*RAS* CRC has resulted in clear detrimental effects. Of those 7% to 10% of patients with mCRC who are *BRAF* V600E–mutated, initial results of combining *BRAF* and MEK inhibitors look promising. The addition of anti-EGFR therapy to overcome feedback activation of the RAS pathway is also being investigated in clinical trials. Similar improvements in efficacy are seen in patients with MMR deficiency who are treated with immunotherapy, as well as those with *HER2* positivity who are treated with targeted anti-HER2 agents.

More recent efforts have been made to classify CRC genetically into different subgroups.[64] However, while these subgroups have important prognostic implications, distinct connections have not been made between these subgroups and molecular predictive markers and targeted therapies.

Taken together, a large percentage of CRCs harbor specific molecular characteristics that define response (or lack of response) to therapy, and thus broad molecular testing has the potential to benefit the vast majority of patients with mCRC. The optimal sequencing of testing has yet to be

defined, but future studies should incorporate broad molecular testing to identify additional patient subgroups, and to understand the optimal time for testing patients.

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