BRAF mutation as a biomarker in colorectal cancer

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Abstract: Nearly 10% of colorectal cancers (CRCs) harbor mutations in BRAF. While cytotoxic chemotherapy remains central to the treatment for patients with metastatic CRC, there is a growing understanding that CRC is comprised of molecularly and clinically distinct populations. BRAF-mutant CRC is one such subset. We are learning more about the complexity of BRAF-mutant CRCs and the ways in which patients with BRAF-mutant CRCs may or may not benefit from targeted therapies. This article reviews the role of BRAF as a biomarker in CRC and its implications for treatment.

Keywords: personalized medicine, colorectal cancer, BRAF

Overview of CRC

In 2015, an estimated 132,700 people will be diagnosed with CRC and nearly 50,000 people will die of this disease. Treatment for stage I, stage II, and stage III disease is curative in intent and includes surgery, adjuvant cytotoxic chemotherapy to prevent recurrent disease for a subset of patients with stage II colon cancers and nearly all patients with stage III CRCs, and radiation for many patients with stage II and stage III rectal cancers. For patients with metastatic or stage IV CRCs that are not surgically resectable, treatment is palliative and the goal is to improve disease-related symptoms and to prolong life. Treatments include systemic chemotherapy with drugs such as 5-fluorouracil, oxaliplatin, and irinotecan, as well as administration of bevacizumab and ziv-aflibercept, which are anti-vascular endothelial growth factor agents. For patients with RAS-wild-type (RAS-WT) tumors, monoclonal antibodies directed at the epidermal growth factor receptor (EGFR), cetuximab and panitumumab, can be added to systemic chemotherapy.

Biology of EGFR/KRAS/BRAF signaling

The molecular landscape in CRC is complex with genetic alterations seen in the genes encoding the RAS family of proteins (KRAS and NRAS), the RAF kinases (ARAF, BRAF, and CRAF), phosphatidylinositol-3-kinase (PI3K), EGFR, human epidermal growth factor receptor 2 (HER2), and others. The most commonly seen and readily targetable alterations center on the EGFR/RAS/RAF signaling pathway. EGFR is a member of the ErbB family of receptor tyrosine kinases. Activation of the transmembrane receptor of EGFR results in autophosphorylation and dimerization of the receptor, activating the RAS family proteins (HRAS, KRAS, and NRAS). This in turn regulates multiple downstream pathways critical for cell survival and proliferation, including, but not limited to, the RAF family of kinases (ARAF, BRAF, and CRAF) and PI3K. The RAF kinases comprise a key regulatory pathway, which then
activates the downstream MEK and ERK pathways. Alterations at nearly each of the kinases listed earlier can be seen in patients with CRCs, and each represents an opportunity to target and personalize therapies.\textsuperscript{3–7}

Biomarkers in CRC

The most widely known biomarkers in CRC are KRAS and NRAS, and 32%–40% and 2%–5% of CRCs, respectively, have alterations in these genes. The vast majority of KRAS alterations are in codons 12 and 13.\textsuperscript{2,8} Activating mutations in RAS cause EGFR-independent, constitutive activation of downstream pathways, resulting in uncontrolled cell survival and proliferation. Consequently, patients with RAS-mutant CRCs do not receive benefit from EGFR-directed therapies.\textsuperscript{2,3–11} EGFR-directed therapies, such as cetuximab and panitumumab administration, have been shown to improve response rate and overall survival only for patients with RAS-WT CRCs.\textsuperscript{2,3–11} Given these treatment implications of the presence or absence of RAS mutations, testing for KRAS and NRAS mutations is standard in the care of patients with metastatic CRCs.

BRAF alterations in cancer

Activating BRAF mutations, most commonly in codon 600, are present in many solid tumors, including CRCs (10%), melanomas (50%), and lung cancers (1%–2%).\textsuperscript{1,2,14,15} Single-agent inhibition of BRAF via vemurafenib or dabrafenib has changed the way we treat patients with melanoma and lung cancer. Sosman et al\textsuperscript{16} performed a single-arm, Phase II study of patients with BRAF-mutant melanoma. These patients with BRAF-mutant melanoma received the BRAF inhibitor vemurafenib and they experienced a median overall survival of 15.9 months and response rates of 53%.\textsuperscript{16} Chapman et al\textsuperscript{17} performed a randomized Phase III study of patients with BRAF-mutant melanoma, in which patients were randomized to receive either vemurafenib or the standard of care at the time, a cytotoxic chemotherapy called dacarbazine. They demonstrated that patients receiving vemurafenib had improved response rates and overall survival compared to patients receiving dacarbazine, with overall survival improving from 9.7 months to 13.6 months.\textsuperscript{17,18} On the basis of these studies, vemurafenib and dabrafenib have been approved for the treatment of patients with BRAF-mutant melanoma. In patients with BRAF-mutant lung cancers, promising case reports of dramatic clinical and radiographic responses, similar to the responses seen in patients with melanoma, have been published.\textsuperscript{19–21} Initial results from a Phase II study\textsuperscript{22} evaluating dabrafenib in patients with BRAF-mutant lung cancers found that 33% (28/84) had a partial response. Although not yet approved in BRAF-mutant lung cancer, dabrafenib has been given a breakthrough designation by the US Food and Drug Administration for consideration of rapid approval in the treatment of patients with BRAF-mutant lung cancers. While single-agent inhibition of BRAF has been promising and effective for patients with melanoma and lung cancers, treatment of BRAF-mutant CRC has been more challenging.

BRAF in CRC

Clinical and pathologic features of patients with BRAF-mutant CRC

Patients with BRAF-mutant CRCs are characterized by unique clinical and pathologic characteristics. Although the absolute numbers in each study are small, several features characteristic of BRAF-mutant colon cancer have become apparent. In the largest retrospective study to date,\textsuperscript{23} mutation status, clinical outcomes, and clinicopathologic features were determined and analyzed in patients participating in the N0147 study. The N0147 study evaluated the role of various adjuvant chemotherapy regimens administered to patients after surgery for stage III (lymph node-positive) colon cancer.\textsuperscript{23} Of 2,166 colon cancers undergoing BRAF testing, 310 (14%) had BRAF V600E mutations. Compared to patients with BRAF-WT tumors, patients with BRAF-mutant colon cancers were more likely to be ≥70 years old (34% vs 14%, \(P<0.001\)), female (63% vs 45%, \(P<0.001\)), white (94% vs 85%, \(P<0.001\)), and a current or former smoker (62% vs 52%, \(P<0.001\)). Patients with BRAF-mutant colon cancers are also more likely to have right-sided tumors (86% vs 48%, \(P<0.001\)). These tumors are also more likely to be characterized by a poorly differentiated or with high-grade histology (47% vs 22%, \(P<0.001\)), and more invasive (14% vs 10%, \(P<0.04\)).\textsuperscript{23} These findings are consistent with other studies on patients with BRAF-mutant colon cancers and their tumors.\textsuperscript{1,4,23–26} In CRC, BRAF mutations have been found to be mutually exclusive with RAS mutations.\textsuperscript{23,27}

Additionally, the pattern of metastatic spread at diagnosis appears unique in this patient population compared to that in patients with BRAF-WT tumors. Liver involvement at the time of diagnosis was less common (60% vs 80%, \(P<0.01\)), and peritoneal involvement was more common (26% vs 14%, \(P<0.01\)).\textsuperscript{26} Axillary lymph node involvement, a highly unusual site of metastatic disease for CRC, has also been seen in patients with BRAF-mutant CRC. In a retrospective review...
of 100 patients with \textit{BRAF}-mutant CRC at Memorial Sloan Kettering Cancer Center, nine patients had axillary lymph node metastasis.\cite{36}

\textit{BRAF}-mutant CRC tumors were also more likely to be deficient in DNA mismatch repair.\cite{37,38} As described by Gon-salves et al.,\cite{39} in the case of 310 patients with \textit{BRAF}-mutant CRCs, 47\% of patients had DNA mismatch repair-deficient tumors, compared to 7\% of patients with \textit{BRAF}-WT tumors ($P<0.001$). Abnormalities in the DNA mismatch repair pathway are identified by assessing for the presence of microsatellite instability or performing immunohistochemical staining for the presence or absence of proteins important in the DNA mismatch repair pathway. While mismatch repair deficiency is often associated with germline-inherited cancer predisposition syndromes, such as Lynch syndrome (hereditary nonpolyposis colorectal cancer or HNPCC), mismatch repair deficiency can also occur secondary to somatic alterations in genes important for DNA mismatch repair. Although the association among \textit{BRAF}V600E-mutant CRC, mismatch repair deficiency, and tobacco history has not been completely clarified, some have argued that tobacco exposure among patients with \textit{BRAF}-mutant CRCs results in increased DNA methylation, subsequent silencing of these methylated genes that may be important in mismatch repair, and, ultimately, development of the molecular and immunohistochemical phenotype of a DNA mismatch repair-deficient tumor.\cite{40}

Consequently, if a DNA mismatch repair-deficient tumor harbors a \textit{BRAF}-V600E mutation, there is virtual certainty that the DNA mismatch repair status is probably secondary to a somatic alteration in the DNA rather than a germline change associated with an underlying cancer predisposition syndrome, and germline testing for Lynch syndrome is therefore not indicated.

**Prognostic implications of \textit{BRAF} mutations**

\textit{BRAF} mutation status is consistently associated with poor prognosis in multiple retrospective evaluations. Using data from the PETACC-3 randomized Phase III trial evaluating the utility of adding irinotecan to 5-fluorouracil/leucovorin for the adjuvant treatment of CRC, the prognostic value of \textit{BRAF} mutations was evaluated.\cite{41} Of 3,278 patients enrolled in this trial, 1,307 had \textit{BRAF} testing performed prospectively. Eight percent (103/1,307) had evidence of \textit{BRAF} mutations. While no difference was seen between patients with \textit{BRAF}-WT and \textit{BRAF}-mutant colon cancers with respect to recurrence-free survival, patients with \textit{BRAF}-mutant, DNA mismatch repair-intact CRC had poorer overall survival, with an increased hazard ratio of 2.2.\cite{42} Additionally, in a retrospective evaluation of 229 patients with metastatic CRCs undergoing treatment with systemic chemotherapy, overall survival was 11 months for patients with \textit{BRAF}-mutant CRCs ($n=15$) compared to 41 months for patients with \textit{KRAS}-WT/\textit{BRAF}-WT tumors ($n=135$).\cite{43} Overall survival for patients with \textit{BRAF}-mutant CRCs is typically measured at less than 1 year.\cite{44,45}

While patients with \textit{BRAF}-mutant CRC clearly have a poorer prognosis, Popovic et al\cite{28} evaluated whether gene expression can clarify the underlying biology and clinical course of patients with both \textit{BRAF}-mutant and \textit{BRAF}-WT CRCs. Using a gene expression classifier incorporating 64 genes, a population of patients with \textit{BRAF}-WT CRCs who had similar gene expression profiles as patients with \textit{BRAF}-mutant CRCs was identified. These patients had similarly poor outcomes, suggesting an underlying and poorly understood biologic similarity between these molecularly distinct patient populations.

**Treatment implications of \textit{BRAF} mutations**

Regarding standard chemotherapy treatment decisions, the presence of \textit{BRAF} mutations in CRC is thought to play no role in the sensitivity of tumors to standard cytotoxic chemotherapy, such as oxaliplatin and irinotecan.\cite{46} In the MRC-FOCUS trial, which compared first-line treatments with 5-fluorouracil, 5-fluorouracil/oxaliplatin, and 5-fluorouracil/irinotecan, patients with \textit{BRAF}-mutant CRCs had a shorter overall survival compared to patients with \textit{BRAF}-WT CRCs; however, no association was seen between the presence of \textit{BRAF} mutations and response to chemotherapy with oxaliplatin versus irinotecan.\cite{47} On the basis of these data, \textit{BRAF} mutation plays no role in the choice of standard, cytotoxic chemotherapy.\cite{48}

Regarding response to standard, targeted therapies, growing evidence suggests that \textit{BRAF} mutations in CRC predict lack of response to EGFR-directed therapies, despite the fact that these CRCs are \textit{RAS}-WT, and EGFR-directed therapies are effective for patients with \textit{RAS}-WT CRCs, as demonstrated in the CRYSTAL trial for cetuximab and in other prospective and retrospective evaluations of the use of EGFR-directed therapies for patients with \textit{RAS}-WT CRCs.\cite{49,50,51,52,53,54,55} There is growing evidence to suggest that \textit{BRAF} alterations predict lack of response or, at a minimum, lack predictive value in relation to \textit{BRAF} mutations and EGFR-directed
therapies. In an evaluation of the CRYSTAL and OPUS trials evaluating the role of cetuximab in the first-line treatment of patients with CRC, BRAF mutations were identified in 70/800 patients, and no significant differences were seen in treatment outcomes for patients with BRAF-mutant and BRAF-WT CRC receiving EGFR-directed therapy.  

De Roock et al evaluated tumor samples from 773 patients with CRC treated with cetuximab, in addition to performing comprehensive genotyping of KRAS, BRAF, NRAS, and PIK3CA. Of 36 patients found to have BRAF-mutant CRC, only 8% of patients responded to treatment (2/24) with cetuximab. In other retrospective studies of patients with metastatic CRC treated with EGFR-directed therapies, patients whose tumors harbored BRAF alterations did not receive benefit from EGFR-directed therapies. These studies, however, are all limited in their retrospective nature and overall small numbers of patients with BRAF-mutant CRC, with each study having between nine and 24 patients with BRAF-mutant CRC receiving EGFR-directed therapy.

To address this issue, several meta-analyses have been undertaken to further understand whether the presence or absence of BRAF mutations can be linked to response to EGFR-directed therapies. One meta-analysis evaluating the role of EGFR-directed therapies in patients with BRAF-mutant CRCs has noted that the response rate to EGFR-directed therapies in BRAF-mutant patients compared to BRAF-WT patients was 0.14 (95% confidence interval [CI]: 0.04–0.53), suggesting that patients with BRAF-mutant CRCs do not respond to EGFR-directed therapy. Similarly, another meta-analysis of nine Phase III trials and one Phase II trial with 463 patients with BRAF-mutant CRCs revealed that the addition of EGFR-directed therapies did not improve response rate, progression-free survival, or overall survival. These findings suggest that the presence of RAS or BRAF mutations in CRC predicts lack of response to EGFR-directed therapies, and we recommend that comprehensive evaluation for the presence or absence of BRAF and RAS alterations should be performed at the time of diagnosis of metastatic disease to determine whether patients will benefit from EGFR-directed therapies.

This recommendation is consistent with that of the National Comprehensive Cancer Network, which “strongly recommends genotyping of tumor tissue in all patients with metastatic CRC for RAS (KRAS exon 2 and non-exon 2; NRAS) and BRAF at diagnosis of stage IV disease.” Historically, testing for molecular alterations in patients with CRC was performed via molecular testing of individual genetic alterations in KRAS. Given the need for molecular analysis of multiple genes, including KRAS, NRAS, and BRAF, more comprehensive molecular testing is needed. Options can include mutation-profiling assays to identify “hotspot” mutations that are seen frequently in these and other oncogenes, using technologies such as Sequenom™ mass spectrometric genotyping and polymerase chain reaction-based assays. Another more comprehensive option is the use of next-generation sequencing technology in targeted panels of important cancer-associated genes, including BRAF, KRAS, NRAS, and hundreds of others, to identify mutations as well as gene amplifications, deletions, and fusions. The clinical significance and treatment implications of many of these findings remain unclear in the standard care of patients with CRC.

Given the poor outcomes in patients with BRAF-mutant CRCs, Yaeger et al sought to understand the role of metastasectomy in these patients. Although resection of limited metastatic disease can provide cures for patients with metastatic CRCs, their data indicated that patients with BRAF-mutant CRCs had a nonsignificant shorter recurrence-free survival after metastasectomy compared to that in patients with BRAF-WT tumors (7 months compared to 11 months, P<0.084) and a significantly shorter overall 2-year survival (61% compared to 86%, P=0.003). Similar findings are noted by Renaud et al, who evaluated 180 patients with metastatic CRC who underwent resection of lung metastases. Patients with BRAF-mutant CRCs were found to have a significantly worse median overall survival of 15 months compared to that of patients with KRAS-mutant and KRAS-WT/BRAF-WT CRCs (55 months and 98 months, respectively). These findings raise concern that metastasectomy in patients with BRAF-mutant CRCs is far less likely to provide the durable responses and cures seen in some patients with BRAF-WT CRCs and, thus, suggest that BRAF mutation may be a relative contraindication to metastasectomy with curative intent.

Targeting BRAF in CRC

Single-agent targeting of BRAF in CRC

Given the remarkable responses for single-agent BRAF inhibitors in patients with BRAF-mutant melanomas and lung cancers, evaluations of single-agent BRAF inhibitors were undertaken in CRC. Disappointingly, no meaningful clinical activity was seen in patients with BRAF-mutant CRCs. In a Phase I study of vemurafenib, 21 patients with BRAF-mutant CRCs were treated. Although the drug was well tolerated, it was essentially inactive, with only one patient having a confirmed partial response. Median progression-free survival was 3.7 months. Five patients did have a mixed...
response, although these findings are certainly less promising than the results of BRAF inhibition and melanoma. These findings suggest that BRAF-mutant CRC is more complex than those other cancers in which single-agent targeting of BRAF is sufficient. Treatment of BRAF-mutant CRC with single-agent vemurafenib cannot be recommended at this time.

In preclinical studies, mirroring the clinical findings, single-agent vemurafenib treatment of BRAF-mutant CRC cell lines yielded only transient inhibition of BRAF due to compensatory feedback activation of EGFR and its downstream pathways such as MEK and ERK. However, combination treatment of these BRAF-mutant CRC cell lines with both vemurafenib and EGFR-targeted drugs such as cetuximab or gefitinib (an EGFR tyrosine kinase inhibitor) resulted in more sustained inhibition of EGFR, BRAF, and its downstream pathways.

Combination strategies targeting BRAF in CRC

On the basis of these laboratory findings, multiple clinical trials have been undertaken, which have demonstrated somewhat more promising evidence of activity after combination therapy targeting BRAF. Yaeger et al recently published findings from a pilot trial in which 15 patients with BRAF-mutant CRCs were treated with a combination of BRAF and EGFR inhibition. Of 12 evaluable patients, disease shrinkage was noted in ten patients, including two with a partial response. These responses, however, have been transient, with median progression-free survival of 3.2 months (95% CI: 1.6–5.3 months). Preliminary findings from another study and a case report evaluating cetuximab and vemurafenib demonstrated similar results.

Preliminary findings from other studies have also noted modest activity with combination therapies targeting EGFR, BRAF, and MEK. Specifically in a Phase I/II trial of the BRAF inhibitor dabrafenib and the MEK inhibitor trametinib, 43 patients with BRAF-mutant CRCs were treated with the combination and 27/43 had disease control with stable disease (n=22), partial response (n=4), or complete response (n=1).

In another study, ten patients with BRAF-mutant CRCs received combination therapy with dabrafenib, panitumumab, and trametinib targeting MEK. Of six evaluable patients receiving this triplet combination, four had partial responses and two had stable disease. Of nine patients with BRAF-mutant CRCs who received combination therapy with dabrafenib and panitumumab, 7/8 evaluable patients had stable disease. These combinations were reasonably well tolerated, with no Grade 4 or 5 events in patients receiving doublet therapy and one event each of >Grade 3 vomiting, rash, and skin fissures, thought at least possibly related to drug. While preliminary results from the aforementioned studies are perhaps more promising than were seen with single-agent BRAF inhibitors, these responses are short lived for most patients.

Mechanisms of resistance to BRAF inhibition in CRCs

Given the transient responses to BRAF-targeted therapies in patients with CRCs, postprogression biopsies have been useful in identifying mechanisms of acquired resistance to BRAF-targeted therapies. Ahronian et al performed whole-exome sequencing on paired pretreatment and posttreatment samples from patients who underwent combination therapy directed at BRAF alterations. In two patients with BRAF-mutant CRCs who received RAF inhibitors and EGFR inhibitors, posttreatment biopsies revealed the emergence of a KRAS amplification in a first paired sample and a BRAF amplification in a second sample. In a third paired sample from a patient with BRAF-mutant CRC treated with a RAF inhibitor and a MEK inhibitor, posttreatment biopsies demonstrated the development of mutations in both MEK1 (F53L) and ARAF (Q489L). In a tumor cell line harboring a MEK1 F53L mutation and also in a tumor cell line derived from this patient’s tumor, treatment with an ERK inhibitor both alone and in combination with a BRAF inhibitor could rescue the cells from resistance to the RAF and MEK inhibitors. Although limited numbers of samples have been tested here, these findings suggest a diverse pattern of acquired resistance mechanisms in patients with BRAF-mutant CRCs treated with agents targeting BRAF, EGFR, and MEK. These alterations, however, all remain centered on the RAS-RAF-MEK-ERK pathway, suggesting that continued inhibition of this pathway through alternate means may be a way to overcome this resistance. These findings also highlight the critical importance of obtaining paired samples and biopsies at the time of resistance on clinical trials of targeted agents.

Future directions

While mutated BRAF has been a powerful target in certain cancers, revolutionizing the way we treat selected patients with melanomas and lung cancers through single-agent use of RAF inhibitors in patients with tumors harboring alterations in BRAF, in CRC, the treatment paradigm of using single-agent targeted therapy in a molecularly selected...
population has been thus far unsuccessful. The biological underpinnings of BRAF-mutant CRC appear more complex. Combination strategies targeting RAF, EGFR, and MEK have been more successful, with improved response rates, although the beneficial effects have been short lived. To better understand the mechanisms of acquired resistance in this population, whole-exome sequencing in paired pre- and posttreatment biopsies has been illustrative, demonstrating molecular alterations in the same pathway and suggesting that combinations and targeting of downstream kinases may rescue tumor cells from resistance.

While much progress has been made in our understanding of this molecular-defined subset of CRC, many unanswered questions remain. First, clinical trials in this population of patients have focused exclusively on the management of patients in the metastatic setting. Can we personalize the adjuvant treatment of patients with early-stage BRAF-mutant CRC to ultimately improve outcomes in a setting when this disease remains curable? Developing better treatment strategies to manage these patients in the early-stage setting is necessary, given that these patients’ outcomes remain poor when they develop metastatic disease.

Second, BRAF-mutant CRCs have an association with mismatch repair-deficient CRCs, and recent data suggest that patients with mismatch repair-deficient CRC have high response rates, with durable responses to immunotherapies. Can patients with BRAF-mutant, mismatch repair-deficient CRC benefit from immunotherapy in this same way? Finally, we have seen promising but short-lived response in patients with BRAF-mutant CRC to targeted therapies. Developing tolerable combination treatment strategies with sustained inhibition against BRAF and its associated pathways will be critical to meaningfully improve the outcomes for patients with this disease. Given the poor overall survival and outcomes for patients with this disease, continued research and efforts into treating this subgroup of patients with CRCs are warranted.

Disclosure
The authors report no conflicts of interest in this work.

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