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SYSTEMIC THERAPIES IN COLORECTAL CANCER (SM KAZMI, SECTION EDITOR)

Systemic Therapy in BRAF V600E-Mutant Metastatic Colorectal Cancer: Recent Advances and Future Strategies

Van K. Morris [1]

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Abstract

Purpose of Review This review seeks to detail the clinical and pathologic features specific to $BRAF^{V600E}$ colorectal cancer. Application of novel preclinical findings translated into the clinic for the development of new therapeutic options for patients with $BRAF^{V600E}$ metastatic colorectal cancer will be detailed.

Recent Findings While BRAF inhibitors as monotherapy do not have the same clinical activity for colorectal cancer relative to other solid tumors harboring an oncogenic $BRAF^{V600E}$ mutation, combination approaches targeting BRAF + MEK + EGFR hold promise for patients with $BRAF^{V600E}$ colorectal cancer.

Summary Simultaneous targeting of multiple drivers along the MAPK pathway improves clinical outcomes for patients with $BRAF^{V600E}$ colorectal cancer. Targeted therapies and immunotherapy hold great promise in the years to come for patients with this subtype of colorectal cancer.

Keywords Colorectal cancer · BRAF · Immunotherapy · Targeted therapy · Clinical trial · Biomarker

Introduction

In 2018, it is estimated that almost 50,000 Americans will succumb to colorectal cancer (CRC) [1]. For the majority of patients presenting with metastatic CRC, systemic chemotherapy remains the mainstay of treatment. However, targeted therapies, personalized to the genomic profile of a given colorectal tumor, have demonstrated clinical benefit for patients whose tumors are wild-type at the *KRAS* and *NRAS* loci (anti-EGFR antibodies) [2], harbor microsatellite instability (immune checkpoint blockade therapies) [3•], or contain an *NTRK* fusion (Trk inhibitor) [4].

BRAF mutations define another distinct molecular subentity of the CRC population in whom remarkable treatment advances have occurred within recent years. These alterations are present in approximately 8–10% of metastatic CRC cases and are most frequently characterized by valine-to-glutamic acid substitutions at codon 600 of the *BRAF* gene [5]. These $BRAF^{V600E}$ mutations activate oncogenic signaling of the MAPK pathway via eventual downstream phosphorylation of ERK and result in heightened proliferative and anti-apoptotic behavior for the tumor cell [6]. This review highlights the relevant clinical advances in the context of the unique underlying tumor biology for patients with metastatic $BRAF^{V600E}$ CRC.

Characterization of $BRAF^{V600E}$ CRC Tumors

Clinically, $BRAF^{V600E}$ mutations have been linked with female gender, history of tobacco exposure, and advancing age [7–9]. Pathologically, they occur more commonly as proximal (right-sided), poorly differentiated, mucinous CRC tumors [9–11]. In addition, $BRAF^{V600E}$ CRC tumors are more likely to have deficient mismatch repair (dMMR) and be classified as MSI-high (MSI-H) [12]. Here, microsatellite instability arises not from a germline mutation in the dMMR genes associated with hereditary non-polyposis colorectal cancer (HNPCC) syndrome but rather from epigenetic silencing derived from promoter hypermethylation of the *MLH1* gene [13, 14]. In general, $BRAF^{V600E}$ CRC tumors are characterized by

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✉ Van K. Morris
vkmorris@mdanderson.org

[1] Department of Gastrointestinal Medical Oncology, University of Texas, MD Anderson Cancer Center, Houston, TX 77030, USA

extensive methylation across the genome not typically observed across other molecular subpopulations of CRC [15].

Hypermethylation across promoter regions of specific gene foci enriched with cytosine-guanine regions, termed CpG-island methylator phenotype (CIMP)–high tumors, drives tumorigenesis in precancerous, dysplastic cells along the sessile serrated adenoma pathway [16, 17]. This pattern of colorectal tumor development, to which $BRAF^{V600E}$ mutations are linked [18–20], is distinct from the traditional adenoma pathway from which the majority of colorectal cancers arise. While an oncogenic driver in many other tumors (including, but not limited to, melanoma, thyroid cancer, and non-small cell lung cancer), the introduction of a $BRAF^{V600E}$ mutation into a normal colorectal cell does not transform the cell into a cancer [21, 22]. Capitalizing on the CIMP-high biology, methylation (and subsequent loss of function) of tumor suppressor genes in a sessile serrated polyp with a preexisting $BRAF^{V600E}$ mutation creates the synergistic interaction necessary to generate a malignant colorectal lesion [23]. Here, the interplay between the complex, coexisting pathogenic drivers parlays into the aggressive clinical phenotype seen with $BRAF^{V600E}$ CRC tumors relative to their *BRAF* wild-type ($BRAF^{WT}$) counterparts.

With regard to genomic alterations, $BRAF^{V600E}$ mutations occur mutually exclusively in colorectal cancer to activating mutations in the *KRAS* and *NRAS* oncogenes [24, 25], which also drive pathogenic signaling of the MAPK pathway. Analysis of 276 CRC specimens by the Cancer Genome Atlas (TCGA) project found that $BRAF^{V600E}$ mutations co-occur with hypermutated tumors that carry higher somatic mutation burdens intratumorally [26]. This association is likely driven by the *MLH1* hypermethylation and reinforces the interplay between the epigenome and corresponding genomic characteristics unique to this subtype. Certainly, an understanding of the biology relevant and specific to $BRAF^{V600E}$ CRC can inform the oncologist on the clinical manifestations in order to optimize treatment planning.

$BRAF^{V600E}$ as a Clinical Biomarker in CRC

$BRAF^{V600E}$ mutations carry a poor prognostic implication for patients with CRC, regardless of the stage at presentation. In the PETACC-3 trial [27], 3278 patients with stage II or stage III CRC were randomized to receive adjuvant chemotherapy with 5-fluorouracil with or without irinotecan. Nearly half of trial participants (1403, or 43%) had archival tissue available for genomic profiling, including *BRAF* mutation status. Overall survival (OS) for patients with microsatellite stable tumors was lower when accompanied by a $BRAF^{V600E}$ mutation for patients with stage II and with stage III disease alike.

Worsened clinical outcomes for patients with $BRAF^{V600E}$ CRC also extend to patients with stage IV tumors [28–31]. For example, one series of 524 patients with metastatic CRC demonstrated a significantly inferior OS for patients with $BRAF^{V600E}$ tumors when compared to patients with $BRAF^{WT}$ tumors (10 versus 35 months, respectively) [7]. When limited to microsatellite stable CRC, one series showed that the risk of cancer-specific mortality was higher in the *BRAF*-mutated group (hazard ratio (HR) 2.3, 95% confidence interval (CI) 1.3–4.0) [32]. Another series, however, demonstrated that, even for patients with MSI-H metastatic CRC, progression-free survival (PFS) with standard chemotherapy (3 versus 10 months, $P < 0.001$) and OS (14 versus 30 months, $P < 0.001$) is shortened for patients with $BRAF^{V600E}$ CRC tumors [33]. Therefore, the worsened prognostic implications linked to $BRAF^{V600E}$ mutations for patients with advanced CRC appear to be consistent regardless of microsatellite status.

Despite these tumors lacking driver mutations in *KRAS* or *NRAS*, there has not been benefit demonstrated with use of anti-EGFR antibodies as a lone targeted therapy for $BRAF^{V600E}$ metastatic CRC. For example, in the PRIME study [34], patients with untreated, advanced CRC were treated with FOLFOX chemotherapy with or without the anti-EGFR antibody panitumumab. Here, there was no benefit with the addition of panitumumab in PFS or in OS despite the patients having $RAS^{wild\text{-}type}$ tumors. A separate study examined tumors from 773 patients with metastatic CRC treated with cetuximab (another anti-EGFR antibody) as part of their treatment for mutations in *KRAS*, *BRAF*, and *PIK3CA*, in order to assess for a correlation with clinical outcomes [35]. Compared to their wild-type counterparts, those with $BRAF^{V600E}$ tumors treated with cetuximab had lower disease control rates (odds ratio (OR) 0.15, $P = 0.001$), shorter PFS (HR 3.7, $P < 0.001$), and shorter OS (HR 3.0, $P < 0.001$). Other series have likewise confirmed that a $BRAF^{V600E}$ mutation does not serve as a predictive biomarker for response to anti-EGFR therapies for patients with metastatic CRC [36•, 37–41]. Given this lack of data demonstrating a clinical benefit, use of anti-EGFR antibodies as a single targeted therapy for $RAS^{wild\text{-}type}$, $BRAF^{V600E}$ metastatic CRC is not recommended.

Because patients with $BRAF^{V600E}$ CRC are expected to fare poorly in the metastatic setting, systemic chemotherapy options have remained limited. The randomized phase III TRIBE trial compared FOLFIRI/bevacizumab to FOLFOXIRI/bevacizumab as frontline therapy for metastatic CRC, where response rates were improved in the overall population by the addition of oxaliplatin to FOLFIRI/bevacizumab [42]. Post hoc stratification reported a non-significant trend in PFS benefit with FOLFOXIRI/bevacizumab uniquely for the $BRAF^{V600E}$ patients relative to the $BRAF^{WT}$ patients (HR 0.57, 95% CI 0.27–1.2) [43]. While encouraging, the numbers of *BRAF*-mutated patients here treated with FOLFIRI/bevacizumab ($N = 12$) or with FOLFOXIRI/bevacizumab ($N = 16$) remain small and limit definitive interpretation

accordingly. In addition, administration of triplet cytotoxic chemotherapy with FOLFOXIRI is associated with greater toxicity. Therefore, these findings should be interpreted with caution and should not yet be generalized to the entire *BRAF*^{V600E} CRC population, given the rapid clinical deterioration often inherent to their underlying aggressive disease that would not allow tolerance of this regimen. Following loss of response to frontline systemic chemotherapy, outcomes for patients with *BRAF*^{V600E} metastatic CRC have been reported to be especially poor. In a single-institution, retrospective series of 72 patients with *BRAF*^{V600E} metastatic CRC [44], median PFS with systemic therapy in the second-line and third-line settings were 2.5 months and 2.6 months, respectively. Alternatively stated, patients with *BRAF*^{V600E} metastatic CRC developed disease progression even by the time of first restaging when treated with standard options beyond the first line of chemotherapy.

Targeted Therapies Against MAPK Signaling in *BRAF*^{V600E} Advanced Cancers

The advent of targeted therapies against MAPK signaling in recent years has heralded in promising new options for patients with *BRAF*^{V600E} advanced cancers. Vemurafenib is a selective inhibitor specific to the mutated BRAF^{V600E} kinase domain [45] which first demonstrated promising clinical activity in patients with advanced *BRAF*^{V600E} melanoma [46], with rapid reductions of tumor burden observed even within 2 weeks of single-agent therapy [47]. The response rate for *BRAF*^{V600E} metastatic melanoma was reported in a large phase III trial [48] at 48%, higher than the 5% of patients with response to dacarbazine in the control arm of the same trial. Survival outcomes here were likewise improved with vemurafenib, a result which led to FDA approval for this BRAF inhibitor. Similarly, patients with *BRAF*^{V600E} or *BRAF*^{V600K} unresectable melanoma participating in a phase III trial of the reversible BRAF^{V600E} kinase inhibitor dabrafenib (versus dacarbazine) showed responses to the former agent in 50% of cases [49]. BRAF inhibitors as monotherapy have anti-tumor activity in other *BRAF*^{V600E} tumors besides melanoma, such as non-small cell lung cancer, thyroid cancer (papillary and anaplastic), hairy cell leukemia, and Langerhans cell histiocytosis [50–53]. Collectively, for various solid tumors, the presence of a *BRAF*^{V600E} mutation serves as a predictive biomarker for clinical benefit with targeted therapies against the BRAF kinase.

While inhibition of the kinase domain of the downstream MEK has also demonstrated clinical efficacy as a single agent in these tumors, the combination of targeted therapies against BRAF and MEK together delays the onset of acquired resistance in preclinical models of *BRAF*^{V600E} tumors [54], relative to BRAF inhibitors as monotherapies. Here, inactivation of mutated BRAF pharmacologically can generate resistant clones by activating mutations in *MAPK1/2* and other drivers in MAPK signaling. These findings have likewise translated into the clinical setting for patients with *BRAF*^{V600E} malignancies. BRAF/MEK combinations with vemurafenib/cobimetinib, dabrafenib/trametinib, and encorafenib/binimetinib have demonstrated clinical benefit in patients with *BRAF*^{V600E} melanoma [55–57], non-small cell lung cancer [58], and anaplastic thyroid cancer [59]. In *BRAF*^{V600E} melanoma, this combination approach has clinical superiority over single-agent BRAF inhibitors and has been associated with response rates in the 55–70% range. Therefore, sustained anti-tumor activity is promoted by dual targeting of BRAF and MEK in order to deepen blockade of the pivotal MAPK signaling and translates to improved clinical outcomes in patients with advanced cancers harboring *BRAF*^{V600E} mutations.

Targeting MAPK Signaling in *BRAF*^{V600E} CRC

Pivoting upon the successes of targeted therapies for BRAF with or without MEK inhibitors, it would be expected that a similar pattern of clinical activity would be observed for patients with *BRAF*^{V600E} metastatic CRC. However, a study of single-agent vemurafenib in 21 patients resulted in a single patient with a radiographic partial response (response rate (RR) 5%, 95% CI 0–26%) [60•]. Median PFS here was 2.1 months among a pretreated population and did not appear to prolong survival outcomes relative to historical controls. Similarly, low response rates with BRAF inhibitors were observed with dabrafenib (RR 11%, 95% CI 0–48%) [61] and with encorafenib (RR 0%, 95% CI 0–23%) [62]. Seemingly, the response rates are lower for patients with colorectal cancer in this monotherapy approach than in other cancers despite harboring the same oncogenic *BRAF*^{V600E} mutation.

Clinical outcomes likewise do not appear to improve with the addition of a MEK inhibitor to a BRAF inhibitor for *BRAF*^{V600E} metastatic CRC. In one trial [63], 43 patients were treated with the combination of dabrafenib and trametinib. Responses were noted in 5% of patients (RR 12%, 95% CI 4–26%), and median PFS was 3.5 months. Again, patients receiving these two agents overall did not fare as well as other patients with *BRAF*^{V600E} tumors receiving the same treatment.

Subsequent basic science work helped to elucidate the lack of response to these agents in a mechanism unique to *BRAF*^{V600E} CRC. In vitro, *BRAF*^{V600E} CRC cell lines are inherently resistant to vemurafenib, but a siRNA screen revealed that sensitivity to this drug could be restored with knockdown of EGFR [64••]. This finding implicated EGFR activation as a culprit responsible for de novo resistance to targeted therapies against BRAF in this setting. Additional work in xenograft models of *BRAF*^{V600E} CRC confirmed an anti-tumor response preclinically with a combination of agents against BRAF and

EGFR simultaneously that was not observed with either protein was targeted alone [65, 66].

These promising preclinical findings led to a next generation of clinical trials testing BRAF and EGFR combination approaches for patients with metastatic *BRAF*^{V600E} CRC. In a phase I trial of vemurafenib, cetuximab, and irinotecan [67], an initial signal was observed, with radiographic responses reported in 6 of 17 patients (RR 37%). In order to build upon these promising early clinical findings, the randomized phase II SWOG 1406 clinical trial was conducted [68••]. Here, 99 patients with pretreated *BRAF*^{V600E} CRC were treated with irinotecan/cetuximab with or without vemurafenib so that the benefit of the addition of a BRAF inhibitor to an anti-EGFR targeted therapy could be tested prospectively in this setting. This study met successfully its primary endpoint, with a prolongation in PFS (4.3 months versus 2.0 months, HR 0.48, $P = 0.001$) for those receiving vemurafenib, irinotecan, and cetuximab. Disease control rates (DCR, defined as the proportion of patients with stable disease or partial/complete response as the best radiographic assessment according to RECIST 1.1 criteria) were also more favorable in this group (67% versus 22%, $P = 0.001$). Subgroup analysis here failed to demonstrate preferential clinical activity based on microsatellite status, sidedness (left versus right) of the primary tumor, or *PIK3CA* mutation status. Overall, this trial provided the first randomized prospective data confirming that the previously detailed preclinical data that targeted approaches against BRAF and EGFR are effective in this population. As a result, the combination of vemurafenib, irinotecan, and cetuximab was updated in the 2018 NCCN Guidelines [69] as a recommended therapy for patients with *BRAF*^{V600E} metastatic CRC.

Other studies investigating this approach in parallel have corroborated this strategy, with similar clinical findings as observed with the SWOG 1406 study. In a single-arm study, 15 patients with *BRAF*^{V600E} metastatic CRC were treated with vemurafenib and panitumumab (no cytotoxic exposure here) [70]. This dual combination was safe and well tolerated. For the 12 patients assessable for response, two patients (RR 13%) had radiographic responses, with one of these participants experiencing a 100% reduction in tumor volume from baseline. Eleven patients (89%) had a radiographic response or stable disease by RECIST 1.1 criteria as their best-measured assessment on the study. Another trial of 20 patients with *BRAF*^{V600E} metastatic CRC treated with dabrafenib and panitumumab observed radiographic responses in 2 patients (RR 10%) [71].

Encorafenib and cetuximab were examined in 54 patients with *BRAF*^{V600E} metastatic CRC treated with ($N = 28$) or without ($N = 26$) alpelisib, a class I PI3K inhibitor [72]. Use of alpelisib here was supported by preclinical data demonstrating in xenograft models of *BRAF*^{V600E} CRC that PI3K/mTOR signaling may drive de novo resistance to BRAF inhibition, and that sensitivity to targeted therapies can be restored by a PI3K inhibitor [66]. Response rates were 18% and 19% for those treated with encorafenib and cetuximab with or without alpelisib, respectively. While this study was not designed statistically to compare the two treatment arms, there appeared to be no added clinical benefit by the addition of a PI3K inhibitor here. Although the total numbers were small, alterations in PI3K/mTOR genes did not correlate with anti-tumor activity.

Extending Clinical Benefit for Patients With *BRAF*^{V600E} Metastatic CRC

Despite the practice-changing successes noted with BRAF and EGFR targeted therapies here, acquired resistance to these drugs invariably develops, a theme unfortunately common across solid tumors. Understanding of resistance mechanisms has led however to further advancements in the management of *BRAF*^{V600E} metastatic CRC. In the aforementioned phase I study of vemurafenib, cetuximab, and irinotecan [67], post-progression blood samples were analyzed for genomic profiling by circulating tumor DNA in order to identify acquired alterations not present in baseline samples which could be implicated in resistance to treatment. Oncogenic aberrations in *KRAS*, *EGFR*, *ARAF*, *MAP2K1*, *GNAS*, and *ERBB2* were all observed in this series of patients and implicated reactivation of MAPK signaling as a driver of tumor progression of targeted therapies against BRAF and EGFR. Addition of inhibitors to downstream effectors of MAPK signaling like MEK and ERK have restored sensitivity in preclinical models of *BRAF*^{V600E} metastatic CRC resistant to BRAF and EGFR targeted therapies [73•, 74] and have provided rationale for triple combination approaches deepening efforts to thwart this oncogenic pathway in this subpopulation of CRC.

In a trial of dabrafenib, panitumumab, and trametinib for 91 patients with *BRAF*^{V600E} metastatic CRC, complete or partial responses were seen in 19 (21%) patients, with a DCR of 86% [71]. Grade 3/4 toxicity profiles were worse than for those receiving dabrafenib and panitumumab without a MEK inhibitor. Median PFS (both ~ 4 months) was likewise similar with the triple combination than dabrafenib and panitumumab. Nonetheless, these findings suggested, based upon the notable DCR, that simultaneous targeting of BRAF, EGFR, and MEK holds promise for this population with poor tumor biology.

Perhaps the most encouraging result to date for the treatment of *BRAF*^{V600E} metastatic CRC has been from the recent report of efficacy from the safety lead-in analysis [75••] of the BEACON trial, a randomized phase III study of irinotecan/cetuximab versus encorafenib/cetuximab with or without binimetinib. In the initial pilot in which 29 patients with *BRAF*^{V600E} metastatic CRC were treated with the BRAF/EGFR/MEK combination, radiographic responses were seen in 14 patients (RR 48%), with all 29 patients having disease control (i.e., no de novo progression at first restaging

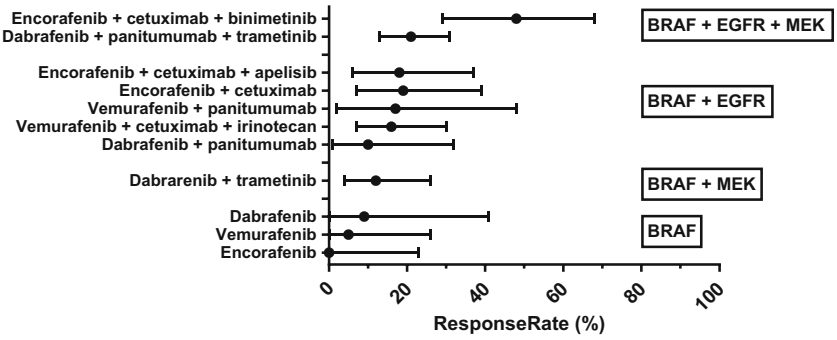

Fig. 1 Evolution of targeted therapies in $BRAF^{V600E}$ metastatic colorectal cancer: response rate (95% confidence interval)

according to RECIST 1.1 criteria) as the best response. Median PFS here was 8.0 months. Final analyses of the phase III trial are highly anticipated. However, these very encouraging early results led to FDA breakthrough therapy designation for the combination of encorafenib, cetuximab, and binimetinib for patients with $BRAF^{V600E}$ metastatic CRC. The development of improved response rates with subsequent generations of clinical trials testing MAPK-targeting agents in this setting are detailed in Fig. 1.

Immunotherapy in $BRAF^{V600E}$ Metastatic CRC

Immune checkpoint blockade agents targeting PD-1/PD-L1 and CTLA-4 have demonstrated durable clinical activity for patients with MSI-H metastatic CRC [3•]. Given the association between microsatellite instability and *BRAF* mutations in patients with CRC, these agents are relevant in this population as well. Indeed, although the numbers of patients treated are small, objective responses to the anti-PD-1 antibody nivolumab as a single agent (RR 25%) [76] and in combination with the anti-CTLA-4 antibody ipilimumab (RR 55%) [77••] for patients with MSI-H, $BRAF^{V600E}$ metastatic CRC. Therefore, immunotherapy is an attractive option with safety and efficacy alike for those patients with MSI-H tumors and coexisting $BRAF^{V600E}$ mutations.

Conclusions

Unique tumor biology specific to $BRAF^{V600E}$ colorectal tumors generate a clinical phenotype vastly different to their $BRAF^{WT}$ counterparts and cause hastened clinical deterioration and poor survival outcomes. That traditional agents for metastatic CRC are futile from an efficacy standpoint in the $BRAF^{V600E}$ CRC setting prompted preclinical efforts implicating activity from targeted therapies targeting MAPK signaling drivers. Most recently, inhibitors of BRAF, EGFR, and MEK have revolutionized how clinicians will approach $BRAF^{V600E}$ metastatic CRC in the years to come. In doing so, the evolving treatment of $BRAF^{V600E}$ CRC provides a real-life success story of translational, bench-to-bedside science.

Compliance With Ethical Standards

Conflict of Interest The author declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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