Molecular targeted therapy of *BRAF*-mutant colorectal cancer

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Abstract: Over the past two decades, the molecular characterization of metastatic colorectal cancer (mCRC) has been revolutionized by the routine implementation of *RAS* and *BRAF* tests. As a result, it is now known that patients with mCRC harboring *BRAF* mutations experience a poor prognosis. Although it accounts for only 10% of mCRC, this group is heterogeneous; only the *BRAF*-V600E mutation, also observed in melanoma, is associated with a very poor prognosis. In terms of treatment, these patients do not benefit from therapeutics targeting the epidermal growth factor receptor (EGFR). In first-line chemotherapy, there are two main options; the first one is to use a triple chemotherapy combination of 5-fluorouracil, irinotecan, and oxaliplatin, with the addition of bevacizumab, because *post hoc* analysis of randomized trials have reported interesting results. The other option is to use double chemotherapy plus bevacizumab, since anti-EGFR seems to have modest activity in these patients. Only a small percentage of patients who experience failure of this first-line treatment receive second-line treatment. Monotherapy with BRAF inhibitors has failed in this setting, and different combinations have also been tested. Using the rationale that BRAF inhibitor monotherapy fails due to feedback activation of the EGFR pathway, BRAF inhibitors have been combined with anti-EGFR agents plus or minus MEK inhibitors; however, the results did not live up to the hopes raised by the concept. To date, the best results in second-line treatment have been obtained with a combination of vemurafenib, cetuximab, and irinotecan. Despite these advances, further improvements are needed.

Keywords: BRAF inhibitors, *BRAF* mutation, chemotherapy, colorectal cancer

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Introduction

Colorectal cancer (CRC) remains one of the main causes of cancer mortality around the world. Although global mortality is decreasing, an increased mortality in young adults (<50 years old) has been reported.[1] Virus-induced rapidly accelerated fibrosarcoma (*v-RAF*) was first identified as an oncogene through the cloning of a viral mouse gene that had the ability to transform NIH3T3 cells. Its human ortholog *CRAF* (RAF-1) and subsequently the related kinase genes *ARAF* and *BRAF* were later found to be commonly mutated in cancer. This RAF kinase family consists of key components of the RAS–RAF–MEK–ERK signaling cascade (MAPK pathway; Figures 1 and 2). The *BRAF* (*v-RAF* murine sarcoma viral oncogene homolog B; B-type raf kinase) gene is located on chromosome 7. Like RAS, the serine/threonine-protein kinase BRAF is a downstream signaling protein in the epidermal growth factor receptor (EGFR)-mediated pathway; *in vitro* experiences have highlighted that some genes are differently expressed in *BRAF*-mutant and wild-type CRC cell lines.[2,3] A characteristic gene expression signature associated with *BRAF* mutation has been identified.[4] However, attempts to directly inhibit the active BRAF protein failed in metastatic CRC (mCRC),[5] suggesting a more complex (or at least different) carcinogenic process in this disease. Nevertheless, *BRAF* mutation testing is now recommended for mCRC in the latest National Comprehensive

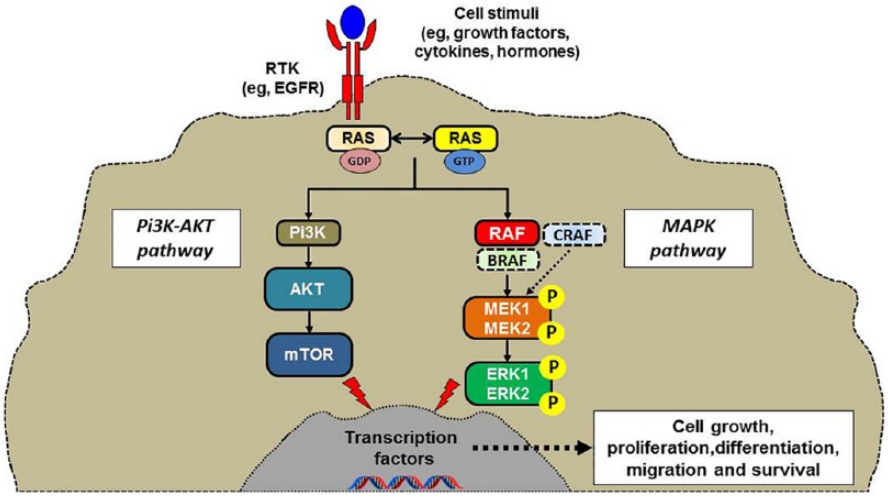

Figure 1. The RAS–RAF–MEK–ERK cellular signaling cascade.

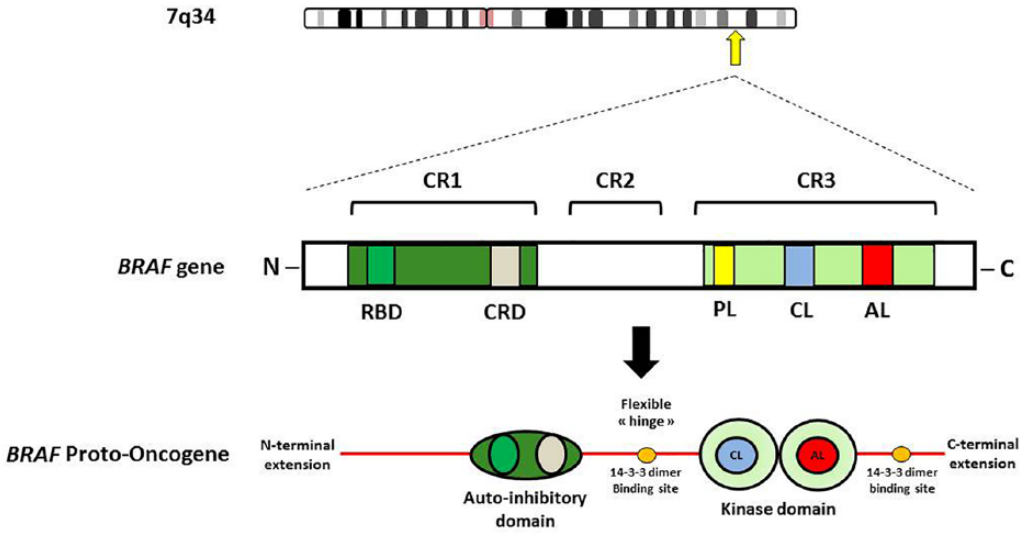

Figure 2. *BRAF* schematic primary structure, showing functional domains.
AL, activation loop; CL, catalytic loop; CR, conserved region; CRD, cysteine-rich domain; KD, kinase domain; P-L, phosphate-binding loop; RBD, RAS-binding domain.

Cancer Network guidelines.[6] We will discuss and review here the more recent literature that specifically concerns *BRAF*-mutant CRC.

The BRAF pathway and the biological consequences of *BRAF* mutation in colorectal cancer carcinogenesis

The MAPK pathway plays a major role in homeostasis of cellular proliferation, differentiation, survival, and apoptosis. *BRAF*-mutant CRC typically harbors a valine to glutamic acid change at codon 600. As for the deleterious *KRAS* mutation such as G12, this alteration in the BRAF kinase domain results in a constitutively active protein. However, *BRAF* mutations in certain disease subtypes, such as hypermethylated right-sided CRC, suggest that additional tumor features and alterations are associated with the presence of *BRAF*-V600E and will determine the final signal output.[7] Although the two genes work closely in the same pathway, the gene expression patterns of *KRAS*-mutant and *BRAF*-mutant mCRC are very different from each other.[8] Furthermore, the oncogenic contribution of mutated *BRAF* may vary between tumor types, as suggested by the very heterogeneous clinical benefit provided by BRAF inhibition treatment strategies in melanoma and mCRC.[5,9]

It has been reported that *BRAF*, and especially V600E mutations lead to constitutive BRAF kinase phosphorylation of MEK and ERK kinases and sustained MAPK pathway signaling. As soon as the RAF kinases are activated, MEK1 and MEK2 are phosphorylated and activated, and as a consequence ERK1 and RK2 are phosphorylated and activated.[10] This ERK activation produces phosphorylation of numerous substrates both in the nucleus and the cytosol, leading to an enhancement of cell proliferation and a longer survival. Despite numerous accessible crystal structures of wild-type *BRAF* and *BRAF*-V600E, the mechanism by which *BRAF*-V600E mutants activate BRAF remains poorly understood. A study of 218 *BRAF*-V600E-mutated colorectal tumors demonstrated a clear heterogeneity within this group of tumors. This identified two distinct subgroups independent of microsatellite instability (MSI) status, *PI3K* mutation, sex, and sidedness.[11] A subset of tumors was characterized by high KRAS/mTOR/AKT/4EBP1/EMT activation, while cell-cycle dysregulation characterized the other. These different subgroups of *BRAF*-V600E mutations may explain the nonuniform responses to drug therapies, including BRAF and MEK inhibitors. Considering the difficulty of developing specific BRAF inhibitors, it is clear that the specific structural mechanism of different *BRAF* mutations still requires further study.

Epidemiology

BRAF mutations are present in 5–15% of CRC, with a higher mutation rate in right-sided colon cancer.[12,13] In a report comprising 2530 patients with mCRC included in three randomized trials (COIN, FOCUS, and PICCOLO), the prevalence of *BRAF* mutations was 9.1%.[14] In a population-based study that could better reflect the true incidence, 12% of the patients had *BRAF*-V600E mutant tumors.[15] In another population-based report the percentage of *BRAF*-mutant tumors was even superior to 20%.[16] Dual mutations of *RAF* and *RAS* genes are rarely seen: 8 among the 2530 patients (0.3%) and 0.01% of cases in another series.[17] There are more *BRAF* mutations in right-sided colon cancer than in left-sided colon cancer. For instance, the SPECTAcolor trial revealed that the percentage of *BRAF*-mutant tumors was 10.5% in the total population of 370 patients, and was 22.6% in patients with right-sided colon cancer *versus* only 5.1% in patients left-sided colon cancer.[17] In a large pooled biomarker analysis evaluating the role of biological markers in defining the prognosis of stage II and III colon cancer beyond TNM classification, a stepwise decrease in the prevalence of *KRAS* or *BRAF*-V600E mutations was observed when moving from right-sided to left-sided colon cancer. *BRAF* mutations (and *KRAS*) were approximately twice as likely to be found in the caecum than the sigmoid colon.[18]

BRAF-mutant tumors: clinical and histopathological specificity

Patients with *BRAF*-mutant CRC are more likely than those with wild-type CRC to be female, have right-sided tumors, or have peritoneal or nodal metastases, but are less likely to have lung metastases. In addition, their tumors more frequently have mucinous histology.[19] The signet ring cell phenotype also seems to be more frequently observed but this could be related to the MSI status also observed in these patients.[20] Classically, *BRAF* mutations are common in sessile serrated adenomas and seem to appear first in this kind of adenomas.[21] In these neoplasms, *BRAF* mutations are associated with MSI, hypermethylation, and minimal chromosomal instability.[22] The association between *BRAF* mutation and MSI in CRC could be related to the relationship with the high-level CpG island methylator phenotype (CIMP) and MLH1 promoter methylation. It has also been suggested that there is an association between current or former smoking history and the presence of *BRAF*-V600E mutations in tumors that could be also related to the CIMP phenotype.[23,24] Although the exact mechanism remains unknown, preclinical studies have shown that tobacco exposure can stimulate the DNA methyltransferase activity that is associated with CIMP.[25]

The patterns of *BRAF*-mutant tumors have been shown to be so specific that a nomogram for predicting mutational status of mCRC has been published.[26] A predictive score was assigned to each of the following variables: the primary site of the tumor, the patient's sex, and the mucinous characteristics of the cancer. The sum of the scores was converted to the probability of *BRAF* mutation occurrence, and was 81% in female patients with mucinous-type right-sided colon cancer.

BRAF-mutant colorectal cancer and microsatellite instability

BRAF mutations are observed in 40–60% of the sporadic CRC harboring high MSI (MSI-high); in contrast, *BRAF* mutations are never seen in patients

with Lynch syndrome.[27] In a metastatic setting, *BRAF*-mutant tumors were more likely to have MSI than wild-type tumors (12.6% *versus* 3.0%, $p < 0.001$).[14] In a pooled analysis on localized colon cancer,[28] the prevalence of *BRAF*-V600E mutations and MSI status paralleled each other, with an increase from the caecum to the hepatic flexure, then a gradual decrease through the sigmoid colon. *BRAF*-V600E mutations were eight times more prevalent in MSI-high than microsatellite stable (MSS) tumors. In all published series, there is an overlap between MSI and *BRAF*-V600E tumors, with a major impact on prognosis (see below). Hence, MSI status should always be included in studies that address *BRAF* mutation status.[29]

Impact of *BRAF* mutations on prognosis

Impact of BRAF mutations on prognosis in an adjuvant setting

A retrospective, pooled biomarker study evaluated 4411 tumors for *BRAF* and *KRAS* mutations and mismatch repair status; 3934 were MSS and 477 were MSI. In MSS patients, all *BRAF*-V600E mutations [hazard ratio (HR): 1.54; 95% confidence interval (CI): 1.23–1.92, $p < 0.001$)], *KRAS* codon 12 alterations, and p.G13D mutations (HR: 1.60; 95% CI: 1.40–1.83, $p < 0.001$) were associated with shorter time to recurrence and shorter survival after relapse (HR: 3.02; 95% CI: 2.32–3.93, $p < 0.001$, and HR: 1.20; 95% CI: 1.01–1.44, $p = 0.04$, respectively). Overall survival (OS) in MSS patients was poorer for patients with *BRAF*-mutant tumors (HR 2.01, 95% CI 1.56–2.57). In the pooled analysis of stage II and III colon cancers, the HR of median OS between *BRAF*-V600E-mutated and nonmutated tumors was around 2,[28] confirming the prognostic role of the *BRAF*-V600E mutation in an adjuvant setting. There is a relationship between *BRAF*-V600E mutation and the classification of primary colorectal cancer according to the gene-expression-based consensus molecular subtypes (CMSs) that has defined four molecularly and clinically distinct subgroups of tumors.[30] In a large Norwegian series of 1197 colorectal cancer (all stages) it was reported that *BRAF*-V600E mutations are enriched and associated with poor prognosis in CMS1 (immune type) MSS tumors.[31]

Impact of BRAF mutations on prognosis in metastatic disease

The mechanism resulting to the poor prognosis of patients with *BRAF*-mutant mCRC is poorly understood. It was rapidly shown that with standard treatment including targeted therapies, the median OS of these patients was around 12 months, much lower than that obtained in *BRAF*-wild-type patients.[32,33] In terms of progression-free survival (PFS), there was no major difference in first-line treatment. However, following progression on first-line chemotherapy, patients with *BRAF*-mutant CRC had a significantly shorter post-progression survival, and only one-third of patients were able to receive second-line treatment *versus* more than 50% in patients with *BRAF*-wild-type mCRC.[14] In a study evaluating 5FU/folinic acid/irinotecan (FOLFIRI) plus panitumumab in a pure second-line setting, patients with *BRAF*-mutant mCRC had a median PFS of 2.5 months and an OS of 4.7 months, compared with a PFS and an OS of 6.9 and 18.7 months, respectively, in patients with *BRAF*-wild-type tumors.[34]

In contrast, *BRAF* mutation did not change the prognosis of patients with MSI-high tumors: in a Finnish population-based series of 762 patients with sporadic CRC, the poor prognostic effect caused by *BRAF*-V600E mutation (multivariate analysis of 1.88, $n = 34$) was overpowered by the favorable effect of MSI in the MSI/*BRAF*-V600E population (HR: 0.83, 60 patients).[15] The same series showed that patients with sporadic MSS/*BRAF*-V600E-mutated rectal tumors had a very poor prognosis.[15] Exceptional cases of double mutations are also associated with a very poor prognosis.[17]

Are all BRAF *mutations the same?*

As stated previously, the mutation typically observed in CRC is a V600E mutation; this mutation has been described in up to 7% of human cancers and can be present in different tumor types, such as melanoma (66% of cases),[7] thyroid cancer (60%),[7] and lung cancers (9%).[35] The V600E mutation accounts for approximately 95% of the activating mutations in *BRAF* in mCRC.[5]

Although V600E has an adverse impact on prognosis, other rarer *BRAF* mutations do not seem to share the same effect.[36] A total of 10 patients with tumors bearing mutations in *BRAF* codons 594 or 596 were identified and compared with 77 and 542 patients bearing *BRAF*-V600E-mutant and *BRAF*-wild-type tumors, respectively. While *BRAF*-V600E-mutant tumors were more frequently right-sided, mucinous, and with

peritoneal spread, *BRAF* 594 or 596 mutant tumors were more frequently rectal, nonmucinous and with no peritoneal spread. The 10 tumors with BRAF 594 or 596 mutations were MSS. Patients with tumors bearing mutations in *BRAF* codons 594 or 596 had an OS (62 months) that appears even better than those of *BRAF*-wild-type tumors, and clearly different from those with *BRAF*-V600E-mutant tumors (12.6 months; HR: 0.36; 95% CI: 0.20–0.64, $p = 0.002$).[36] In a sample of patients from the Memorial Sloan Kettering Cancer Center with mCRC who had next-generation sequencing performed on their tumor specimens, non-V600E mutations made up approximately 20% of all *BRAF*-mutant tumors. These tumors appear sensitive to EGFR inhibitors.[37] These data have been confirmed by more recent studies: in a total of 9643 patients with mCRC analyzed with next-generation sequencing 208 (2.2%) patients with (non-V600) *BRAF* mutations were identified (22% of all *BRAF* mutations identified).[38] When compared with tumors with *BRAF*-V600E mutations cancers with (non-V600) *BRAF* mutations were found in patients who were significantly younger (58 *versus* 68 years, respectively), who were less frequently female patients (46% *versus* 65%, respectively), and who had fewer high-grade tumors (13% *versus* 64%, respectively) or right-sided primary tumors (36% *versus* 81%, respectively). Median OS was significantly longer in patients with (non-V600) *BRAF*-mutant metastatic CRC compared with those with both (V600E) *BRAF*-mutant and wild-type *BRAF* metastatic CRC (60.7 *versus* 11.4 *versus* 43.0 months, respectively; $p < 0.001$). There is heterogeneity even within *BRAF*-V600E-mutant tumors, and a prognostic score has been built using data from 395 patients. The global score took into account 18 variables; a simplified score restricted to 11 variables has been proposed.[39] Both scores require validation in another series of patients.

Treatment of *BRAF*-mutant tumors

First-line treatment

BRAF mutation and efficacy of anti-EGFR. While data suggest that *BRAF* mutation status has clear prognostic value in mCRC, the predictive value of *BRAF* mutation status for response and benefit from EGFR-directed treatments, such as cetuximab, remains controversial. Retrospective analyses of recent trials have suggested that *BRAF* mutations are not predictive of outcome with EGFR-directed therapies,[40–42] whereas other analyses have suggested that cetuximab and panitumumab are more active in patients with *BRAF*-wild-type mCRC.[43,44] A systematic review and meta-analysis of randomized controlled trials evaluated the effect of *BRAF* mutations on the treatment benefit from anti-EGFR therapy for mCRC.[45] The HR for an OS benefit with anti-EGFR treatment was 0.97 (95% CI: 0.67–1.41) for mutant tumors compared with 0.81 (95% CI: 0.70–0.95) for *BRAF*-wild-type tumors (*RAS* wild-type). However, the test of interaction was not statistically significant. The HR for a PFS benefit with anti-EGFR therapy was 0.86 (95% CI: 0.61–1.21) for *BRAF*-mutant tumors compared with 0.62 (95% CI: 0.50–0.77) for *BRAF*-wild-type tumors (test of interaction, $p = 0.07$). The authors concluded that there was insufficient evidence to state that anti-EGFR therapy has no effect in *BRAF*-mutant tumors.[45] Another meta-analysis, published the same year, did not show any benefit in favor of the use of cetuximab or panitumumab in *BRAF*-mutant tumors (HR for OS: 0.91, NS).[46] It seems at least that the effect is small; for example, the FIRE3 study reported a low median OS of 12.3 months in patients with *BRAF*-mutant tumors who received FOLFIRI + cetuximab as first-line treatment.[47]

BRAF *mutation and efficacy of bevacizumab.* In the first study reporting major efficacy of bevacizumab,[48] median OS was 16 months when patients with a *BRAF*-mutant tumor received bevacizumab *versus* 8 months when they received chemotherapy alone. However, the number of patients included in this *post hoc* analysis was very small (10 patients).[49] In the VELOUR study,[50] 30 patients had *BRAF*-mutant tumors; among them, 11 patients receiving aflibercept and FOLFIRI had a median OS of 11 *versus* 5 months in the 19 patients receiving only chemotherapy.[51] The FIRE3 study, comparing FOLFIRI + bevacizumab or cetuximab, included 48 patients with *BRAF*-mutant tumors.[47] Median PFS was 4.9 months in the patient group receiving cetuximab and 6.0 months in the group of patients receiving bevacizumab (HR: 0.87, NS). Median OS also showed a small nonsignificant advantage in favor of FOLFIRI + bevacizumab (13.7 *versus* 12.3 months). The large United States (US) trial comparing bevacizumab with cetuximab in the first-line treatment of mCRC reported a better median OS for patients with *BRAF*-mutant tumors treated with bevacizumab (median = 15 months in 41 patients) than

in patients treated with cetuximab (median = 11.7 months in 31 patients), but this difference did not reach significance (adjusted HR: 0.61; 95% CI: 0.35–1.06).[52]

FOLFOXIRI + bevacizumab as first-line treatment of BRAF*-mutant tumors: a standard of care?* Despite a lack of evidence to back up the interest in the use of bevacizumab in combination with chemotherapy, the most interesting data obtained to date in *BRAF*-mutant mCRC resulted from a combination of triplet chemotherapy with bevacizumab. Following the results of the first large phase III trial of the GONO group, it has been known for 10 years that triplet chemotherapy with 5FU, folinic acid, irinotecan, and oxaliplatin (FOLFOXIRI) was able to improve efficacy to FOLFIRI in an all-comers patient population.[53] The same group evaluated the role of this combination plus bevacizumab. They reported a very good response rate (90%), median PFS (12.8 months), and OS (30.9 months) in a subgroup of 10 patients with *BRAF*-mutant tumors treated with FOLFOXIRI + bevacizumab (*post hoc* analysis).[54] A prospective study performed in 214 patients that included 15 with *BRAF*-mutant tumors confirmed these results, finding a median PFS and OS of 9.2 and 24.1 months, respectively.[55] When retrospective and prospective results were pooled, median PFS and OS were 11.8 and 24.1 months, respectively.[55] These data have been confirmed by a subgroup analysis of the TRIBE trial, which showed that the 16 patients with *BRAF*-mutant tumors treated with FOLFOXIRI + bevacizumab in this randomized trial had a median OS of 19.0 months, whereas the 12 patients treated with FOLFIRI + bevacizumab had a shorter median OS of 10.7 months.[56] Following these results the consensus European Society for Medical Oncology (ESMO)-Asian guidelines recommended triplet chemotherapy plus bevacizumab as the standard of care for first-line treatment of *BRAF*-mutant CRC.[57]

More recently, a phase II randomized trial showed that addition of panitumumab to the same FOLFOXIRI combination gave an advantage, even in patients with *BRAF* mutations, in terms of response rate: 71% *versus* 22% when compared with chemotherapy. However, there was no difference in median PFS (6.5 and 6.1 months with and without panitumumab, respectively).[58]

On the other hand, due to the weak level of evidence, it can be also suggested another option using FOLFOX bevacizumab in the first line followed by an active second-line combination of irinotecan + cetuximab + vemurafenib recently presented[59] that we will discuss later.

Treatment of BRAF*-mutant tumors after failure of first-line therapy*

Table 1 shows the targeted therapies and treatment of *BRAF*-mutant mCRC. Surprisingly, it has been reported that although fewer patients with *BRAF*-mutant tumors receive second-line treatment, *BRAF* mutation is not associated with inferior second-line outcomes.[14] The first attempt to treat *BRAF*-mutant mCRC used the evident potential resource that was BRAF inhibitors, which had proved to be very effective in the treatment of *BRAF*-V600E-mutant melanoma.[9]

BRAF *mutation and efficacy of RAF inhibitors.* There are many different BRAF inhibitors.[66] Only one compound in the first generation of BRAF inhibitors has obtained approval for the treatment of cancer. This compound, sorafenib, has been tested in the treatment of *KRAS*-mutant CRC in combination with irinotecan[67] but not in *BRAF*-mutant mCRC. The main representatives of the second generation of BRAF-specific inhibitors are vemurafenib, dabrafenib, and encorafenib. Vemurafenib was initially tested as a single agent in a total of 21 *BRAF*-mutant mCRC patients, 19 of whom were evaluable for response. Among these 19 patients, there was only 1 partial response (5%) and the median PFS was 3.7 months.[5] These results were confirmed when no objective response occurred in a basket study evaluating vemurafenib alone in 10 patients.[68] Encorafenib is active in naïve and pretreated melanoma, suggesting that this drug could be effective in mCRC.[69] The third generation of BRAF inhibitors has been developed to fight against the two main mechanisms of resistance that have been described.[70] It means that some of these compounds will be effective and equipotent inhibitors of dimeric forms, as well as monomeric forms of BRAF and that the other type of third-generation BRAF inhibitors, acting as pan-RAF inhibitors, will not induce RAF paradoxical activation.[71]

However, even if monotherapy using new generation BRAF inhibitors could produce better results, it seems that combination regimens are likely to work better than monotherapy in these aggressive tumors in which complex signaling pathways are active.

Table 1. Targeted therapies and treatment of *BRAF*-mutant metastatic colorectal cancer.

Reference	Patients, *n* (type of study)	Treatment	ORR (%)	PFS (months)
RAF inhibitor monotherapy				
Kopetz and colleagues[5]	21 (phase II)	Vemurafenib	5	2.1
RAF inhibitor + MEK inhibitor combination therapy				
Long and colleagues[60]	43 (phase I/II)	Dabrafenib + trametinib	12 (one CR)	3.5
RAF inhibitor + anti-EGFR combination therapy				
Kopetz and colleagues[59]	27 (phase I/II)	Vemurafenib + cetuximab	23	3.7
Das Thakur and Stuart[61]	15 (phase I/II)	Vemurafenib + panitumumab	13	3.2
Prahallad and colleagues[62]	20 (phase I/II)	Dabrafenib + panitumumab	10	3.5
Corcoran and colleagues[63]	26 (phase Ib)	Encorafenib + cetuximab	19 (one CR)	3.7
Schirripa and colleagues[64]	50 (phase II)	Encorafenib + cetuximab	22	4.2
MEK inhibitor + anti-EGFR combination therapy				
Prahallad and colleagues[62]	31	Trametinib + panitumumab	0	2.6
Triple combination therapy				
Prahallad and colleagues[62]	91	Dabrafenib + trametinib + panitumumab	21 (one CR)	4.2
van Geel and colleagues[65]	54 (Randomized phase II)	Vemurafenib+ irinotecan + cetuximab	16	4.4
Schirripa and colleagues[64]	52 (phase II)	Encorafenib + cetuximab + alpelisib	27	5.4

CR, complete response; EGFR, epidermal growth factor receptor; ORR, objective response rate; PFS, progression-free survival.

Combination of targeted therapies

Double combinations. There is convincing nonclinical evidence that robust inhibition of MAPK signaling is needed to more effectively treat *BRAF*-mutant tumors.[72,60] Cancer cells with *BRAF* mutations are highly dependent on MEK/ERK signaling. As demonstrated in melanoma cells, *MEK*-dependent activation of MAPK signaling occurs following BRAF inhibition and near-complete inhibition of phospho-ERK is required for tumor responses.[73] The combination of a BRAF inhibitor and a MEK inhibitor has been

shown to be more active than either agent alone, presumably due to delay or prevention of resistance.[61] In a larger study, a total of 43 patients with *BRAF*-V600E-mutant mCRC were treated with dabrafenib (a BRAF inhibitor) plus trametinib (a MEK inhibitor); 17 of them were enrolled onto a pharmacodynamic cohort undergoing mandatory biopsies before and during treatment. Of 43 patients, 5 (12%) achieved a partial response or better, including 1 (2%) complete response, with a duration of response greater than 3 years; 24 patients (56%) achieved stable disease as best confirmed response. All nine evaluable during-treatment biopsies had reduced levels of phosphorylated ERK relative to pretreatment biopsies.[63]

On the other hand, nonclinical work in CRC cells has shown that BRAF inhibition causes a rapid feedback activation of EGFR that supports continued proliferation of *BRAF*-V600E-mutant tumor cells.[73,62] These reports suggest that activation of EGFR may partially explain the limited therapeutic effect of BRAF inhibitor monotherapy in patients with *BRAF*-V600E-mutant mCRC and that this could be overcome with concomitant EGFR inhibition. However, in the VE-BASKET study, the combination of vemurafenib and cetuximab did not substantially improve the efficacy: there was an objective response rate of 15% in 26 patients.[68] Similarly, when vemurafenib was combined with panitumumab in 15 patients, of whom 12 were evaluable for response; partial responses were observed only in 2 (13%) patients.[74] Encorafenib has been combined with cetuximab and gave a 19% objective response rate in 26 patients.[65]

Triple combinations. Targeting both potential mechanisms of resistance to BRAF inhibitors requires evaluation of the efficacy of triple combinations of a BRAF inhibitor, a MEK inhibitor, and an EGFR inhibitor. A clinical trial that evaluated the combination of dabrafenib, trametinib, and panitumumab in 91 patients reported confirmed complete and partial response in 19 patients (21%) and stable disease in 59 (65%; disease growth control: 86%).[75] Despite these results, it was considered that the proof of concept of the activity of this quite toxic and very expensive triplet combination schedule was not obtained; development of this combination in this indication of *BRAF*-mutant tumors was abandoned. It has also been suggested that PI3K pathway activation could explain resistance to RAF inhibitors in *BRAF*-mutant mCRC. Thus, in parallel with the evaluation of the encorafenib and cetuximab combination already discussed, a triple combination with the addition of alpelisib, a PI3K-alpha inhibitor, has been tested.[65] The objective response rate observed in this phase I study was similar to that with the double combination: 18%. The duration of response was short at 12 weeks, but median PFS was 4.2 months, slightly higher than in the double combination group. Although it does not appear that this triple combination is highly effective, it will be evaluated further.

In another phase Ib/II study that included 19 patients with *BRAF*-V600E mutant tumors, vemurafenib, at doses of 480 mg, 720 mg, and 960 mg twice daily, was combined with panitumumab and irinotecan. Of 17 response-evaluable patients, responses were observed in 6 (35%) patients with a median duration of response of 8.8 months and median PFS of 7.7 months. The most common adverse events observed included fatigue (89%), diarrhea (84%), rash (74%), nausea (74%), anemia (74%), and myalgia (53%).[76] A recently reported randomized phase II study provides additional data.[59] Patients with *BRAF*-V600E mutant tumors were randomized to receive either vemurafenib, irinotecan, and cetuximab every 2 weeks or irinotecan and cetuximab alone. The study included 106 patients and had PFS as its main endpoint. The median PFS was 4.4 months in the patient group receiving the triple combination with vemurafenib *versus* 2.0 months in the patient group treated with the standard doublet alone.[59] A large phase III trial that only includes patients with *BRAF*-V600E mutant tumors has been launched; this trial aims to evaluate the safety and efficacy of the combination of encorafenib (a BRAF inhibitor) plus binimetinib (a MEK inhibitor) given with the anti-EGFR antibody cetuximab in patients with *BRAF*-V600E-mutant mCRC after one or two prior regimens (BEACON CRC trial). The first safety analysis of this phase III trial has been recently reported.[77] First efficacy results will probably be presented in 2019.

Other options. Data from *in vitro* experiments suggested that patients with *BRAF* mutations may have sensitivity to microtubule inhibitors such as vinorelbine.[78] RANBP2 (also known as NUP358) is a small GTP-binding protein belonging to the RAS superfamily that is a crucial regulator of nucleocytoplasmic transport. Suppression of RANBP2 results in mitotic defects only in *BRAF*-like CRC cells, leading to cell death. RANBP2

silencing reduces microtubule outgrowth from the kinetochores, thereby inducing spindle perturbations, providing an explanation for the observed mitotic defects. Thus, *BRAF*-like CRC cells had greater sensitivity to the microtubule poison vinorelbine both *in vitro* and *in vivo*, which suggested that this drug could be an effective treatment for *BRAF*-mutant CRC. Unfortunately, clinical prospective studies did not confirm these preliminary data: a small prospective series of 20 patients reported no objective response, a median PFS of 1 month, and a median OS of 2.1 months.[79]

Toxicity of BRAF inhibitors: a major concern?

Despite the poor prognosis of this patient population, the toxicity profile of drugs must be considered. Common adverse events associated with dabrafenib and vemurafenib include skin toxicities, arthralgia, fatigue, headache, pyrexia, and gastrointestinal events.[80] The incidence of major side effects is not significantly different between dabrafenib and vemurafenib; however, photosensitivity and worsening of liver function tests have been more frequently associated with vemurafenib[81] while pyrexia has been more frequently observed with dabrafenib.[82] The most common skin toxicities associated with BRAF inhibitors have included rash, alopecia, dry skin, hyperkeratosis, pruritus, photosensitivity, hand–foot syndrome. Furthermore, promotion of both benign and malignant hyperproliferative squamous cutaneous lesions has been reported in patients treated with BRAF inhibitors.[83] An increase in the incidence of secondary primary melanoma has also been suggested.[84]

Other events associated with vemurafenib have included QT interval prolongation and worsening liver function test results. QT interval prolongation with vemurafenib is considered rare, liver function abnormalities are usually asymptomatic, but liver injury leading to functional impairment has been reported.[81] Preliminary data suggest also that patients treated with BRAF inhibitors for long periods of time have an increased risk of developing hyperplastic gastric polyps and colonic adenomatous polyps.[85]

BRAF *mutations in MSI-high patients: a completely different therapeutic challenge*

In the first report on the efficacy of pembrolizumab in MSI-high patients, only one patient had a *BRAF*-mutant tumor,[86] preventing any specific conclusion about the efficacy of the programmed cell death (PD)-1 immune checkpoint inhibitor; however, subsequent studies included more MSI-high patients and analyzed their overall results according to their *BRAF* status. A study evaluating the role of nivolumab monotherapy in 74 MSI-high patients reported a response rate of 25% in the 12 patients with *BRAF*-mutant tumors, 27% in the 26 patients with *KRAS*-mutant tumors, and 41% in the 29 patients with both *BRAF*- and *KRAS*-wild-type tumors;[87] there was no statistically significant difference in disease control rate (75, 62, and 78%, respectively). A combination of nivolumab + ipilimumab gave similar results without any influence of *BRAF* mutations on efficacy parameters in MSI-high patients: a 55% objective response rate and 79% disease control rate in 29 patients with *BRAF*-mutant tumors, and a 55% objective response rate and 80% disease control rate in the total population of 119 patients.[88] It can therefore be concluded that in the population of MSI-high patients, *BRAF* mutation status does not have any predictive value in determining the efficacy of PD-1 immune checkpoint inhibitors. All these patients share the same standard of care incorporating immunotherapy into their treatment.

BRAF *mutations detected with circulating tumor DNA*

In mCRC, circulating tumor DNA (ctDNA) analysis could help to determine a subgroup of patients who have *BRAF*-mutant tumors but are classified as *BRAF* wild-type. This false statement can be due to a missed detection in the tumor due to spatial heterogeneity (or therapeutic pressure, that is, temporal heterogeneity). In one study, many more mutations were found by ctDNA analysis than tumor-tissue analysis: 59%, 11.8%, and 14.4% of patients were found to have *KRAS*-, *NRAS*- and *BRAF*-mutant tumors, respectively, by ctDNA analysis compared with 44%, 8.8%, and 7.2% by tumor-tissue analysis.[89] In addition, it has been demonstrated that ctDNA has a higher sensitivity than the lactate dehydrogenase test to detect disease progression, including non-RECIST (Response Evaluation Criteria In Solid Tumors) progression events in melanoma patients.[90] However, there are no data available in patients with mCRC.

Is it possible to propose the same locoregional strategy in patients with BRAF*-mutant than in patients with* BRAF*-wild-type tumors?*

The first study of surgery for patients with liver metastases secondary to *BRAF*-mutant tumors suggested that the risk of appearance of liver metastases is higher in these patients and that OS is poorer after liver resection of their metastases.[91] Schirripa and colleagues confirmed this report, demonstrating in a series of 309 patients undergoing liver resection with tumors biologically assessed for *RAS* and *BRAF* mutations that patients with *BRAF*-mutant tumors ($n = 12$) had a shorter recurrence-free survival: 5.7 months, *versus* 11.0 and 14.4 months for *RAS*-mutant ($n = 160$) and *RAS*-wild-type ($n = 137$), respectively.[64] The same group reported that after response to FOLFOXIRI + bevacizumab, resected patients shared the same prognosis, with a median disease-free survival of 11–12 months independent of their *BRAF* status.[92] However, this retrospective study included only seven patients with *BRAF*-mutant tumors.

Recent cohorts have given slightly discordant results. In a French cohort,[93] 66 patients underwent resection for *BRAF*-mutant liver metastases of mCRC. A case-matched comparison was made with 183 patients who underwent resection for *BRAF*-wild-type liver metastases of mCRC during the same period. The 1- and 3-year disease-free survival rates were respectively 46% and 19% in *BRAF*-mutant and 55% and 28% in *BRAF*-wild-type patients ($p = 0.430$). However, the 1- and 3-year OS rates after surgery were 93% and 54% in *BRAF*-mutant and 96% and 83% in *BRAF*-wild-type patients ($p = 0.004$). The median survival after disease progression was shorter in patients with *BRAF*-mutant tumors.

In a large US cohort including 1497 patients who had complete resection and a known *BRAF* status, 35 (2%) patients had *BRAF*-mutant tumors; of these, 71% had the V600E mutation. Compared with patients with *BRAF*-wild-type tumors, patients with *BRAF*-mutant tumors were older and appeared to have more advanced disease in the liver (more major hepatectomies, for instance) but less extrahepatic disease. Median OS was 81 months for patients with *BRAF*-wild-type tumors and 40 months for patients with *BRAF*-mutant tumors ($p < 0.001$). Median recurrence-free survival was 22 and 10 months for patients with *BRAF*-wild-type and *BRAF*-mutant tumors, respectively ($p < 0.001$). However, long-term survival was possible; it was associated with node-negative primary tumors, CEA $\leqslant 200\,\mu g/l$, and a clinical risk score < 4.[94] A multivariate analysis of a smaller cohort of 849 patients, including 43 (5%) patients with *BRAF*-mutant tumors, revealed that the presence of a *BRAF*-V600E mutation but not a non-*BRAF*-V600E mutation was associated with significantly poorer OS.[95] As a conclusion, these data show that results of liver surgery are poorer in patients with *BRAF*-V600E tumors but that this is still the only hope of cure for these patients. However, considering the aggressiveness of *BRAF* mutation, it can be stated that surgery has to been done as soon as possible when a therapeutic response allowing resection with a hope of cure is reached.

For the future

BRAF-V600E mutant mCRC are insensitive to RAF inhibitor monotherapy due to the feedback reactivation of receptor tyrosine kinase signaling. Combined RAF and EGFR inhibition exerts a therapeutic effect, but resistance invariably develops through undefined mechanisms. As stated previously, currently approved RAF inhibitors inhibit RAF monomers but not dimers. Mechanisms of resistance converge on the formation of RAF dimers, and inhibition of EGFR and RAF dimers could effectively suppress ERK-driven growth of resistant CRC in the future.[96] If their activity is confirmed in clinical trials, third-generation RAF inhibitors that have been selected to have this kind of effect should gain market approval in the future. Pan-RAF inhibitors currently undergoing evaluation are also interesting, because they have been synthesized to be 'paradox breakers', by not inducing RAF paradoxical activation.

Conclusion

Patients with *BRAF*-V600E-mutant mCRC clearly have a poor prognosis and constitute a specific group, making up around at least 10% of all patients with mCRC. FOLFOXIRI + bevacizumab is a possible option; however, the level of evidence-based medicine of this approach remains low. The role of the addition of anti-EGFR to FOLFOXIRI is not yet determined. Beyond the first line, despite the failure of RAF inhibitor monotherapy, some second-line treatments, such as the combination of vemurafenib, irinotecan, and cetuximab, have shown activity. Thus, a sequential use of FOLFOX bevacizumab followed by irinotecan, vemurafenib, and cetuximab is the other valid option. The rapid acquisition of

resistance, either due to dimerization problems, or to the activation of parallel pathways, needs to be addressed to improve the efficacy of RAF inhibitors. The third generation of RAF inhibitors is under investigation and could revolutionize the landscape of mCRC management. Surgery with curative intent is less potent in the treatment of these tumors than in *BRAF*-wild-type tumors, but remains useful and should be proposed as soon as possible after a response to first-line therapy is seen. The challenge of treating MSI-high patients with *BRAF* mutations is completely different, because they respond to immunotherapy in the same way as patients with *BRAF*-wild-type tumors. New drugs, new combinations, and new targets are urgently required in this disease.

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