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Review

Encorafenib plus cetuximab for the treatment of *BRAF-V600E*-mutated metastatic colorectal cancer

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Javier Ros, Nadia Saoudi, Iosune Baraibar, Francesc Salva, Josep Tabernero and Elena Elez

***Abstract*:** B-type *RAF* (*BRAF*)-*V600E* mutations in metastatic colorectal cancer (mCRC) have been described in up to 12% of the patients. This mutation confers a bad prognostic and poor response with standard chemotherapy. Unlike the scenario for *BRAF* mutant melanoma, successful BRAF blockade in mCRC has emerged as a complex path, primarily due to the complex underlying biology of mCRC. The BEACON trial has reshaped the therapeutic landscape of BRAF mCRC demonstrating the benefit of the BRAF inhibitor encorafenib in combination with the anti-epidermal growth factor receptor cetuximab. This paper aims to review the main features of *BRAF* mCRC as well as to review the development of targeted therapy and biomarkers in this specific population. Finally, a deep insight into the underlying biology and molecular classification of *BRAF-V600E* mCRC has also been performed. The words 'BRAF-V600E mutation', 'colorectal cancer', 'BRAF inhibitors', 'consensus molecular subtypes', 'encorafenib', and 'cetuximab' were used to identify the clinical trials from phase I to phase III related to the development of BRAF inhibitors in this population. A deep search among international meetings (American Society of Clinical Oncology and European Society of Medical Oncology) has been performed to incorporate the last trials presented. *BRAF-V600E* mCRC is a challenging disease, mostly because of its molecular biology. The BEACON trial has been the most important therapeutic change in the last decade. Nevertheless, new information regarding biomarkers or novel combinations including BRAF inhibitors plus immune checkpoint inhibitors are also promising.

Keywords: BRAF mutation, cetuximab, colorectal cancer, consensus molecular subtype, encorafenib, transcriptomic signatures

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Correspondence to:
Elena Elez
Department of Medical Oncology, Vall d'Hebron Institute of Oncology, Centro Cellex, Carrer de Natzaret, 115-117, 08035 Barcelona, Spain
meelez@vhio.net

Javier Ros
Department of Medical Oncology, Vall d'Hebron Institute of Oncology, Barcelona, Spain

Medical Oncology, Department of Precision Medicine, Università degli Studi della Campania Luigi Vanvitelli, Naples, Italy

Nadia Saoudi
Iosune Baraibar
Francesc Salva
Josep Tabernero
Department of Medical Oncology, Vall d'Hebron Institute of Oncology, Barcelona, Spain

Introduction

Worldwide, colorectal cancer (CRC) is the third most common cancer and the second highest cause of cancer-related mortality in the United States with almost 150,000 new cases and around 53,000 deaths per year. CRC is a highly heterogeneous disease characterized by multiple genetic alterations with a range of prognoses, and with different responses to targeted agents.[1,2] In recent years, substantial advances have been made regarding personalized treatments in metastatic CRC (mCRC). New agents targeting the B-type *RAF* (*BRAF*)-*V600E* mutation, *HER2* amplification, the *KRAS G12C* mutation, and microsatellite instability (MSI) have all proved successful in certain sub-populations. For most patients with mCRC, cytotoxic chemotherapy with 5-fluorouracil, leucovorin, and oxaliplatin (FOLFOX) and 5-fluorouracil, leucovorin, and irinotecan (FOLFIRI) in combination with monoclonal antibodies [anti-epidermal growth factor receptor (EGFR) or anti-vascular endothelial growth factor (VEGF)] remains the backbone of care, for upfront therapy at a minimum. The RAS/RAF/

MEK/ERK pathway has been particularly implicated in the pathogenesis of mCRC. *BRAF* alterations are driver mutations leading to constitutive activation of the BRAF kinase and sustained MAPK/ERK signaling, resulting in increased cell proliferation, spread, and cancer cell survival. In the particular case of *BRAF-V600E* mCRC, outstanding advances have been made in the last few years despite that this mutation is associated with a poor prognosis and lack of response to standard chemotherapy compared to *BRAF* wild-type counterparts. Unlike the scenario for *BRAF* mutant melanoma, successful BRAF blockade in mCRC has emerged as a complex path, primarily due to the complex underlying biology of mCRC. Here, we review the most relevant clinical trials leading up to the first phase III trial, the BEACON trial, that successfully demonstrated the utility of BRAF blockade with encorafenib plus cetuximab in *BRAF-V600E* mCRC. We also review novel ongoing therapeutic approaches and potential predictive and prognostic biomarkers.

The molecular landscape of *BRAF-V600E* mutations in CRC

BRAF mutations in mCRC have been described in 8–12% of these patients, and exon 15 T1799A transversion resulting in a valine amino acid substitution, is the most frequent alteration (95% of these cases). This leads to constitutive activation of the BRAF kinase resulting in cancer progression. This mutation is associated with poor prognosis, with a median overall survival (OS) of 11 months, and poor response to standard chemotherapy.[3–5] Colorectal tumors with *BRAF-V600E* mutation exhibit a well-defined phenotype; they are more frequent among older females, in right-sided mucinous tumors, and are associated with nodal and peritoneal metastases. From a molecular perspective, this mutation is nearly always mutually exclusive with *KRAS* mutations, while 30% present MSI.[6,7] The *BRAF-V600E* mutation is associated with the CpG island methylation phenotype (CIMP) which leads to hypermethylation of DNA promotor regions and gene silencing. In the case of *BRAF-V600E* CRC, the CIMP phenotype is associated with MSI due to silencing of the *MLH1* promoter gene caused by hypermethylation, leading to a sporadic MSI phenotype.[6,8]

Before the development of BRAF inhibitors, upfront treatment recommendations for *BRAF-V600E* mutant patients came from subgroups of several trials evaluating different chemotherapy regimens. The phase III TRIBE trial compared bevacizumab combined with either 5-fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) or FOLFIRI in the first-line setting.[9] In the subgroup analysis of the 28 *BRAF-V600E* mutant patients, the FOLFOXIRI plus the anti-VEGF bevacizumab was more active than FOLFIRI plus bevacizumab [median OS, 19.0 *versus* 10.7 months and median progression-free survival (PFS), 7.5 *versus* 5.5 months in the triple and double combinations, respectively]. However, these results were not confirmed either in the TRIBE-2 trial[10] or in a subsequent individual patient meta-analysis.[11] The TRIBE-2 trial randomized patients to receive first-line FOLFOX plus bevacizumab followed by FOLFIRI plus bevacizumab after disease progression, or FOLFOXIRI plus bevacizumab followed by the re-introduction of the same regimen after disease progression [PFS-2 hazard ratio (HR) 1.23, 95% confidence interval (CI), 0.72–2.09, $p=0.153$; OS HR, 1.35, 95% CI, 0.79–2.30, $p=0.155$]. Based on these data, there is currently insufficient evidence supporting the use of the triplet cytotoxic regimen over doublet chemotherapy in front-line treatment of *BRAF-V600E*-mutated mCRC, and the recommendation of FOLFOXIRI-bevacizumab should be individualized.

In the second-line setting, the phase III VELOUR trial, a prospective, randomized, double-blind study, evaluated the efficacy and safety of another anti-VEGF combination, comparing aflibercept plus FOLFIRI *versus* placebo plus FOLFIRI in patients with mCRC experiencing disease progression on or after completing an oxaliplatin-based regimen. Analysis of the 36 *BRAF-V600E* mutant CRC patients gave an OS of 10.3 months with FOLFIRI plus aflibercept.[12]

Monoclonal antibodies targeting EGFR have also been tested in the *BRAF-V600E* population. The presence of *BRAF-V600E* mutations has been proposed to be a predictive marker for limited response to anti-EGFR therapies in mCRC patients.[13–16] Furthermore, a meta-analysis including nine phase III trials that compared cetuximab or panitumumab, and involving 463 *BRAF* mutant patients, demonstrated that the addition of an anti-EGFR agent to standard therapy did not increase the benefit, for either PFS (HR, 0.88; 95% CI, 0.67–1.14; $p=0.33$) or OS (HR, 0.91; 95% CI, 0.62–1.34; $p=0.63$).[17] These

findings support *BRAF* mutation assessment before initiation of treatment with anti-EGFR monoclonal antibodies. The phase III CRYSTAL trial evaluated the addition of cetuximab to FOLFIRI. Sub-analysis of *BRAF-V600E* patients showed that in this population the addition of cetuximab did not result in a statistically significant benefit in terms of PFS or OS.[18] Similar results were reported in a retrospective analysis of the FIRE-3 study, in which patients were randomly assigned to either FOLFIRI plus cetuximab or FOLFIRI plus bevacizumab. Analyses did not confirm an anti-EGFR benefit among *BRAF-V600E* mCRC patients.[19] Thus, currently, an anti-VEGF in combination with chemotherapy is preferred over chemotherapy plus an anti-EGFR for patients with *BRAF-V600E*-mutated CRC, for both the upfront and refractory settings. Guidelines on the use of anti-EGFR therapies currently mandate expanded *RAS*/*BRAF* testing and that patients with *BRAF-V600E* mutations should not receive an anti-EGFR either alone or in combination with chemotherapy.[20] While other treatments are recommended in the refractory CRC setting, such as trifluridine/tipiracil or regorafenib, there are no published analyses regarding the activity of these treatments in the *BRAF-V600E* subgroup.[21,22] Promising preclinical data showed synthetically lethal activity of mitotic spindle poisons on *BRAF*-mutated and *BRAF*-like CRC models.[23] Based on these data, a phase II trial tested the activity of vinorelbine in patients with *BRAF-V600E* mCRC. However despite the encouraging preclinical data, the study did not show the signs of clinical activity among the 20 enrolled patients, with an overall response rate (ORR) of 0%, while median PFS and OS were 1.0 and 2.1 months, respectively.[24]

Although V600E is the most frequent *BRAF* mutation in mCRC, several other mutations have been described. A landmark study categorized these mutations based on their oncogenic activity and their ability to activate the ERK pathway.[25] Three different classes of *BRAF* mutations were described. Class I mutations present with significantly increased kinase activity and operate as monomers. They include *V600E, V660K, V600D, V600M*, and *V600R BRAF* mutations.[26] Class II mutations require dimerization with other BRAF oncoproteins leading to a homodimer, are able to activate the ERK pathway without RAS activation, and have an intermediate degree of kinase activity. They include *L597Q*/*R*/*S*/*V, G464V*/*E, G496A*/*V*/*R, K601 E*/*NT*, and *P367 L*/*S BRAF* mutations.[27] Class III include those mutations with the lowest kinase activity, and function in a RAS-dependent manner. These mutations generate dimerization with cRAF, leading to increased ERK activity. They frequently co-occur with *RAS* mutations, and include *D594G, D594N, G466E, and G466V*. Non-*V600E BRAF* mutations occur less frequently, representing less than 5% of all *BRAF* mutations. Non-*V600E* mutations confer similar prognosis as *RAS*/*BRAF* wildtype and are more likely to occur with concomitant *RAS* mutations. Some reports suggest that non-*V600E* BRAF tumors might benefit from anti-EGFR therapies.[7]

Transcriptomic classifications

Two subtypes of *BRAF-V600E* mCRC tumors have been described in terms of gene expression profile and regardless of MSI status, methylation patterns, *PI3CA* mutational status, sidedness, or gender.[5] *BRAF-V600E* mutant subtype 1 (BM1) represents 30% of all *BRAF-V600E* mutant CRC tumors and is characterized by *KRAS*/*AKT* pathway activation, mTOR/4EBP1 deregulation, and epithelial–mesenchymal transition. BM1 also exhibits a strong immune profile (IL2/STAT5/IL6/JAK/STAT3 pathway activation, enriched angiogenesis, and tumor necrosis factor-alpha signaling). The BM1 subtype has a poorer prognosis compared to BM2 subtypes, albeit non-significant, in terms of OS (HR, 1.61; 95% CI, 0.91–2.86; $p = 0.106$) and relapse-free survival (HR, 1.66; 95% CI, 0.95–2.92; $p = 0.076$). BM2 represents 70% of all *BRAF-V600E* mutant CRC tumors and is characterized by dysregulation of the cell cycle and cycle checkpoints. BM2 tumors are enriched in metabolic processes and display high cyclin-dependent kinase 1 and low cyclin-D1 levels.

Also based on transcriptomic classifications, the consensus molecular subtypes (CMS) establish four CRC subgroups.[28] Most *BRAF-V600E* tumors are included in the CMS1 subtype, which are hypermutated, MSI, and immune-infiltrated. These transcriptomic classifications may help explain differences in response to targeted treatments and identify the potential mechanisms of resistance.

The path toward successful BRAF blockade in CRC

The historical evolution of the management of patients with *BRAF-V600E* mutant mCRC

leading to current treatment recommendations and the main contributing studies are summarized in Table 1 and Figure 1 presents the therapeutic targets used in the treatment of this disease.

Initial steps using BRAF inhibitors as monotherapy

After the impressive results seen with BRAF inhibitors in *BRAF-V600E* mutant melanoma patients, this quickly led to the investigation of the potential role of BRAF inhibition in CRC. The results were unexpectedly poor. In the extension cohort that included mCRC in the phase I trial, 21 *BRAF-V600E* mCRC patients with central confirmation by Taq polymerase chain reaction (PCR) were treated with vemurafenib (PLX4032). Only one patient (5%) experienced an objective tumor response, while seven cases of stable disease (SD) lasting at last 8 weeks were reported.[29] Vemurafenib was generally well tolerated, with three patients presenting dose-limiting toxicities (DLT) of grade 3 rash and grade 3 nausea. The clinical activity reported in previously treated *BRAF-V600E*-mutated mCRC was noticeably more modest than that seen in melanoma, suggesting that BRAF activation in mCRC is more complex and requires more in-depth molecular understanding than in melanoma.

Encorafenib (LGX818) is a second-generation highly selective ATP-competitive small molecule RAF kinase inhibitor. Encorafenib monotherapy was evaluated in patients with *BRAF-V600E* mutant refractory mCRC during the dose-expansion part of study CLGX818X2101.[30] Modest clinical activity was observed. A total of 18 patients with mCRC were treated, with an ORR of 5.6% and a disease control rate (DCR) of 67%. Three patients had DLT, one patient presented arthralgia and myalgia, one had insomnia and myalgia, and a third patient had bone pain and vomiting.

The basket trial evaluating vemurafenib for non-melanoma tumors with a *BRAF-V600E* mutation was a first-in-kind clinical trial to recruit patients based on the presence of a molecular alteration rather than on a specific indication. In total, 122 patients with *BRAF-V600E* mutant non-melanoma tumors received single-agent vemurafenib. The mCRC cohort included 10 patients, none of whom had a clinical response.[31] The most common adverse events (AEs) across all patients

Table 1. Main clinical trials and outcomes of targeted therapy in *BRAF-V600E* mutant mCRC.

Clinical trial	Phase	Setting	Patients and treatment	Outcomes			References
				ORR (%)	Median PFS (months)	Median OS (months)	
Monotherapy							
Vemurafenib	I	Pts refractory *BRAF-V600E* mCRC	21 pts vemurafenib 960 mg BID	5	3.7	NA	Kopetz *et al.*[29]
Encorafenib	I	Pts refractory *BRAF-V600E* mCRC	18 pts encorafenib 300 or 450 mg	0	4	NA	Gomez-Roca *et al.*[30]
Vemurafenib	II	All solid tumors *BRAF*-V600 mut	10 pts refractory *BRAF-V600E* mCRC vemurafenib 960 mg BID	0	4.5	9.3	Hyman *et al.*[31]
Dual therapy							
Vemurafenib–cetuximab	II	All solid tumors *BRAF*-V600 mut	27 pts refractory *BRAF-V600E* mCRC vemurafenib 960 mg BID + cetuximab 400 mg	4	3.7	7.1	Hyman *et al.*[31]

(Continued)

Table 1. (Continued)

Clinical trial	Phase	Setting	Patients and treatment	Outcomes			References
				ORR (%)	Median PFS (months)	Median OS (months)	
Vemurafenib–panitumumab	I	Pts refractory *BRAF-V600E* mCRC	15 pts panitumumab 6 mg/kg + vemurafenib 960 mg BID	13	3.2	7.6	Yaeger *et al.*[32]
Dabrafenib–trametinib	I/II	Pts refractory *BRAF-V600E* mCRC	43 pts dabrafenib 150 mg BID + trametinib 2mg QD	12	3.5	NA	Corcoran *et al.*[33]
Dabrafenib–panitumumab	I	Pts refractory *BRAF-V600E* mCRC	10 pts dabrafenib 150 mg BID + panitumumab 6 mg/kg	10	3.5	13.2	Corcoran *et al.*[34]
Encorafenib-cetuximab	Ib/II	Pts refractory *BRAF-V600E* mCRC	50 pts encorafenib 200mg QD + cetuximab 400 mg	22	4.2	NA	Tabernero *et al.*[35]
Binimetinib–encorafenib	Ib/II	Pts refractory *BRAF-V600E* mCRC	11 pts encorafenib 450 mg QD + binimetinib 45 mg BID	18	11	NA	Sullivat *et al.*[36]
Encorafenib–cetuximab	III	Pts with *BRAF-V600E* mCRC ⩾2 prior regimens	220 pts encorafenib 300 mg QD + cetuximab 400mg	20	4.2	8.4	Tabernero *et al.*[37]
Triple therapy							
Encorafenib–cetuximab–alpelisib	Ib/II	Pts refractory *BRAF-V600E* mCRC	52 pts encorafenib 200 mg QD + cetuximab 400 mg + alpelisib 300 mg QD	27	5.4	15.2	Tabernero *et al.*[35]
Dabrafenib–panitumumab–trametinib	I	Pts refractory *BRAF-V600E* mCRC	91 pts dabrafenib 150 mg BID + panitumumab 6 mg/kg + trametinib 1.5 mg QD	21	4.2	9.1	Corcoran *et al.*[34]
Encorafenib–cetuximab–binimetinib	III	Pts with *BRAF-V600E* mCRC ⩾2 prior regimens	224 pts ENC 300 mg QD + cetuximab 400 mg iv + binimetinib 45 mg QD	26	4.3	9	Tabernero *et al.*[37]
Encorafenib–cetuximab–binimetinib	II	Previously untreated *BRAF-V600E* mCRC pts	41 pts ENC 300 mg QD + binimetinib 45 mg BID + cetuximab 400 mg	50	4.9	NA	Grothey *et al.*[38]
Vemurafenib–irinotecan–cetuximab	II	Pts refractory *BRAF-V600E* mCRC	49 pts irinotecan 180 mg/m^2 Q2W + vemurafenib 960 mg QD + cetuximab 500 mg/m^2 Q2W	17	4.2	9.6	Kopetz *et al.*[39]

BID, twice daily; C, cetuximab; ENC, encorafenib; iv, intravenous; mCRC, metastatic colorectal cancer; mOS, median overall survival; mPFS, median progression-free survival; mut, mutation; NA, non-available; ORR, overall response rate; pts, patients; QD, once daily; Q2W, once every 2 weeks.

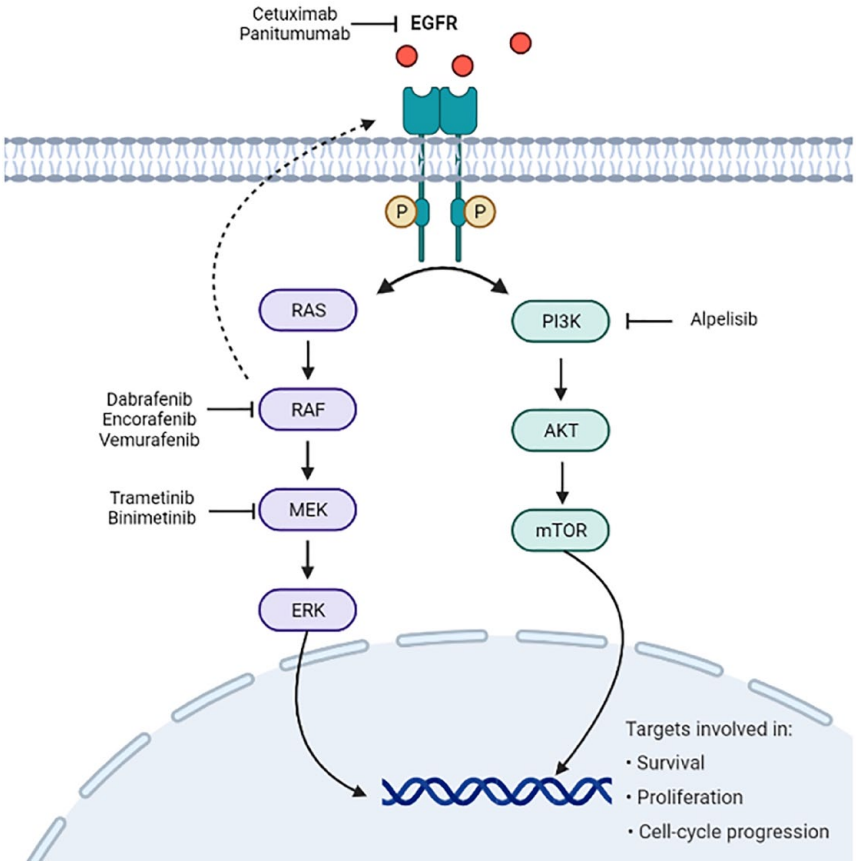

Figure 1. Therapeutic targets used in the treatment of *BRAF-V600E*-mutated mCRC.
mCRC: metastatic colorectal cancer.

receiving vemurafenib monotherapy were rash (68% of patients), fatigue (56%), and arthralgia (40%).

Addition of an anti-EGFR to a BRAF inhibitor improves clinical outcomes

In vitro experiments demonstrated that inhibition at a single node in the MAPK pathway in *BRAF-V600E* mutant CRC cell lines resulted in increased EGFR phosphorylation by adaptative feedback, thus increasing the resistance to the BRAF inhibitor.[40] Interestingly, anti-EGFR therapy rendered these cell lines sensitive to the BRAF inhibitor.[41] In light of the poor clinical results with single agent vemurafenib and this intriguing preclinical evidence, the mCRC cohort of the basket study was amended to include a vemurafenib–cetuximab combination. A total of 27 patients with mCRC *BRAF-V600E* received vemurafenib in combination with cetuximab.[31] One patient had a partial response (PR) giving an ORR of 4%, and 69% of patients presented SD. Median PFS and OS were 3.7 months (95% CI, 1.8–5.1) and 7.1 months (95% CI, 4.4 to not reached), respectively. Patients included in this trial were heavily pretreated having received a median of two lines of previous therapy, ranging from one to six prior lines. Another study evaluated the combination of panitumumab and vemurafenib in 15 *BRAF-V600E*-mutated pretreated patients with mCRC. In all, 10 patients experienced tumor regression, with PR in two patients and SD lasting over 6 months in two patients.[32] Four patients (20%) presented grade 3 or 4 alkaline phosphatase elevations, and one patient (7%) presented neutropenia. Given the success of the dabrafenib and trametinib (a MEK inhibitor) combination in *BRAF* mutant melanoma, combined BRAF plus MEK inhibition was hypothesized to be a promising approach in *BRAF-V600E* mutant CRC. This was implemented using a

combination of dabrafenib plus trametinib, and 43 patients were treated.[33] Of them, five patients (12%) achieved a response, including one complete response (CR) lasting for 36 months. Left ventricular ejection fraction decrease occurred in eight patients (19%), including two grade 3 events, and led to dose reduction in five patients (12%) and treatment discontinuation in one patient (2%). Another trial explored the use of dabrafenib plus the anti-EGFR monoclonal antibody panitumumab with or without trametinib.[34] CR or PR was achieved with dual EGFR/BRAF blockade in 2 out of 20 (10%) *BRAF-V600E* mutant mCRC patients with a median PFS of 3.5 months. The addition of a MEK inhibitor trametinib to the dual EGFR/BRAF blockade improved outcomes, with responses in 9 of 35 patients (26%) and a median PFS of 4.1 months. While both dual and triple blockade showed promising activity, the combination of trametinib and panitumumab gave no responses, whereas toxicity was increased. For patients who received dabrafenib plus panitumumab, no DLT was observed, with grade 3–4 hypokalemia being the main AE. Patients who received trametinib plus panitumumab in the absence of dabrafenib presented significant dermatologic toxicity (18% grade 3–4 dermatitis acneiform).

Promising results were observed in a dose escalation trial with encorafenib and cetuximab in 26 patients with *BRAF-V600E* mutant CRC exploring the combination of encorafenib and cetuximab with or without alpelisib, a phosphoinositide 3kinase (PI3K) inhibitor. In the phase II dose-expansion part of the study, for the 50 patients treated with the encorafenib plus cetuximab combination, median PFS was 4.2 months (95% CI, 3.4–5.4) and the ORR was 22% (95% CI, 12–36).[35] Grade 3–4 AEs presenting in more than 10% of patients receiving doublet treatment were anemia (6%), hyperglycemia (2%), and increased lipase (18%). The combination of binimetinib with encorafenib as dual or triple combination therapy was investigated in a dose-finding phase Ib/II study of binimetinib in combination with encorafenib in patients with *BRAF-V600E* mutant solid tumors. In all, 11 patients were enrolled in a phase II *BRAF* mutant CRC cohort, which had an ORR of 18% (95% CI, 2–52) and a DCR of 64% (95% CI, 31–89). The most frequently reported grade 3 or 4 AEs during the phase II part of the study for the overall population were increased alkaline phosphatase (9% of patients).[36]

The development of the triplet combination

In vitro evidence suggested activation of the PI3K/AKT pathway as another possible mechanism of resistance to *BRAF-V600E* inhibitors.[42] To overcome this, the previously mentioned phase Ib/II study investigated the BRAF inhibitor encorafenib and the anti-EGFR antibody cetuximab with or without the PI3Ka inhibitor alpelisib (BYL719) in patients with advanced *BRAF-V600E* mutant mCRC. The phase Ib study did not identify a maximum tolerated dose for either combination. Based on the general tolerability of the triplet, the phase II encorafenib dose was chosen for both arms. In the phase II part, patients with advanced *BRAF*-mutated CRC failing at least one prior line of therapy were randomized 1:1 to doublet [encorafenib 200 mg once daily (QD) and cetuximab per label] or triplet (encorafenib, cetuximab, and alpelisib 300 mg QD) therapy. A total of 102 patients were randomized (triplet, $n=52$; doublet, $n=50$). A comparison of the triplet *versus* the doublet in terms of efficacy showed an HR of 0.69 (95% CI, 0.43–1.11; $p=0.064$) with a median PFS of 5.4 months (95% CI, 4.1–7.2) and 4.2 months (95% CI, 3.4–5.4), respectively, and confirmed ORRs of 27% and 22%, respectively. With 35 events, an interim OS analysis (triplet *versus* doublet) demonstrated an HR of 1.21 (95% CI, 0.61–2.39). Grade 3 or 4 AEs in the triplet arm were anemia (17%), hyperglycemia (13%), and increased lipase (8%).

The combinations of dabrafenib plus panitumumab, dabrafenib and trametinib plus panitumumab, and trametinib plus panitumumab were also explored. Analyses showed an improved response for the triple therapy compared to either doublet, albeit with an increase in some AEs, notably dermatologic and grade 3–4 diarrhea relative to the doublet regimens.[34] Combinations of targeted therapies with irinotecan, such as cetuximab plus vemurafenib and irinotecan, or irinotecan and cetuximab with or without vemurafenib, have been tested with very modest efficacy results.[39,43] In the phase II S1406 trial, patients with refractory *BRAF-V600E* mCRC were randomized to receive irinotecan plus cetuximab with or without vemurafenib. In this trial, MSI tumors were not excluded because at the time, immune checkpoints inhibitors had not yet received Food and Drug Administration (FDA) approval in this population.[39] Median PFS was 4.2 and 2.0 months in the experimental and control arms, respectively, and the ORR was 17 *versus* 4% ($p=0.05$), with a DCR of 65 *versus* 21% ($p=0.001$),

respectively. In all, 21 patients in the control arm (42%) crossed over to the experimental regimen after disease progression. OS was not significantly different between the two arms (HR, 0.77; 95% CI, 0.50–1.18; $p = 0.23$). PFS following crossover in this cohort was 5.4 months, with an ORR and DCR of 19% and 76%, respectively.

This investigation into a deeper suppression of the BRAF pathway using a triplet blockade combination ultimately led to the development of the BEACON trial. The BEACON trial was an open-label, global, randomized phase III trial for patients with *BRAF-V600E* mCRC who had progressed on at least one previous line.[37] It is the largest trial including *BRAF-V600E* mCRC published to date. Patients were randomly assigned 1:1:1 to receive triplet (224 patients, encorafenib plus cetuximab plus binimetinib), doublet (220 patients, encorafenib plus cetuximab), or control treatment (221 patients, irinotecan-based chemotherapy plus cetuximab). Primary endpoints were OS and independently reviewed ORR comparing the triplet to control treatment. Updated data demonstrated a median OS of 9.3 months (95% CI, 8.2–10.8) for the triplet compared to 5.9 months (95% CI, 5.1–7.1) in the control group (HR, 0.65%; 95% CI, 0.47–0.75).[37] Median OS for the doublet was 9.3 months (95% CI, 8.0–11.3; HR *versus* control was 0.61, 95% CI, 0.48–0.77). Confirmed ORRs were 26.8% (95% CI, 21.1–33.1) for the triplet, 19.5% (95% CI, 14.5–25.4) for the doublet, and 1.8% (95% CI, 0.5–4.6) for control. The study was not powered to compare the triple therapy *versus* doublet treatment. The toxicity profile demonstrated that treatment was globally well-tolerated and consistent with previously reported data, with grade $\geq$3 AEs in 66%, 57%, and 64% for triplet, doublet, and control, respectively. These results led to FDA and European Medicines Agency (EMA) approval for the doublet combination of encorafenib and cetuximab for patients with mCRC with *BRAF-V600E* mutation who have already progressed on at least one prior treatment regimen. Consequently, the BEACON trial has completely reshaped the therapeutic landscape of *BRAF-V600E* mCRC and is currently a new standard of care in this population.

MAPK reactivation as the main mechanism of resistance

Given the modest clinical activity with BRAF inhibitors as monotherapy, mechanisms of primary resistance are suspected. *In vitro* studies, showing the MAPK pathway as a driver of resistance, are confirmed by *in vivo* studies demonstrating that BRAF inhibitors result in increased EGFR phosphorylation. This increases the insensitivity to the BRAF inhibitor, explaining the mechanisms of primary resistance to a single MAPK node blockade. Interestingly, anti-EGFR therapy rendered these cell lines sensitive to the BRAF inhibitor.[40,41] BRAF inhibitors combined with EGFR inhibitors resulted in synergistic inhibition of tumor growth in *BRAF-V600E*-mutated CRC xenograft models.[40,41] Nevertheless, although these combinations induce tumor regression, acquired resistance invariably appears, leading to tumor progression. Most of the trials reviewed here included per protocol analyses of paired biopsies and plasma samples. However, since most patients presented with surgically unresectable disease and given the disease aggressivity with metastases frequently not suitable for biopsy, tumor tissue is not always available for analysis. Matched biopsies before and at the time of progression from eight patients included in several trials evaluating different combinations of BRAF and EGFR inhibitors, revealed genetic amplification of wild-type *RAS* as a recurrent mechanism of resistance, leading to increased receptor tyrosine kinase-dependent activation. Thus, inhibiting *EGFR* and *RAF* dimers offers a potential strategy to overcome resistance in *BRAF-V600E* mutant CRC.[44]

The trial exploring the combination of dabrafenib plus panitumumab with or without trametinib included serial circulating tumor DNA (ctDNA) determination before, during, and at progression using digital PCR BEAMing (Beads, Emulsion, and Magnetics). Plasma levels of *BRAF-V600E* correlate with tumor response.[34] Almost half of the patients (48%) showed emergence of *KRAS* or *NRAS* mutations in ctDNA at the time of disease progression. Similarly, when ctDNA was evaluated following treatment with encorafenib and cetuximab with or without alpelisib, samples collected during acquired resistance showed MAPK activation (*KRAS* mutations or amplifications).[45] The phase S1406 trial evaluating irinotecan plus cetuximab with or without vemurafenib also collected plasma samples. The *BRAF-V600E* mutant allele fraction declined in 87% of patients after treatment initiation, whereas no patients in the chemotherapy arm had *BRAF-V600E* mutation allele fraction decrease. Plasma analysis upon

progression showed one acquired *KRAS* mutation without other identifiable genomic mechanisms of resistance.[39]

In a recent publication, genomic profiling, tumor mutational burden (TMB), and BM transcriptional subtype classification were evaluated as a mechanism of resistance among a small cohort of patients with *BRAF-V600E*/microsatellite stable (MSS) mCRC who received encorafenib with cetuximab, with or without binimetinib. There were no differences between BM or genomic profiling subtypes. The results suggested that high TMB (cutoff six mutations per megabase) limited the benefit from EGFR/BRAF blockade. However, the sample size was modest and these results require prospective validation.[46]

Finally, clonal expansion of *MET* gene amplification subclone during panitumumab and vemurafenib treatment thought to cause tumoral progression has also been described. Interestingly, acquired *MET* amplification was overcome by combining BRAF and MET inhibition with subsequent rapid tumoral response.[47] Based on the reviewed evidence, the majority of acquired mechanisms of resistance are associated with MAPK pathway reactivation *via* alternative pathways.

New scenarios and future approaches for managing *BRAF-V600E* mCRC

The promising preliminary efficacy data from the safety lead-in part of the BEACON trial supported the step toward first-line treatment with encorafenib, binimetinib, and cetuximab in patients with *BRAF-V600E* mutant mCRC. This triplet treatment was explored in the phase II single-arm ANCHOR-CRC trial which included 40 patients.[38] The ORR was 50% with a DCR of 85%, and median PFS was 4.9 months (95% CI, 4.4–8.1). Grade 3 or higher AEs occurred in 68% of patients; the most common grade $\geq$3 AEs were diarrhea (15%), anemia (2%), and nausea (7%). Considering the outcomes with chemotherapy plus anti-VEGF in the setting of upfront therapy, the results from the ANCHOR trial were not as good as expected. Currently, the BREAKWATER trial in *BRAF-V600E* mutant CRC is evaluating the role of the combination of cetuximab and chemotherapy with encorafenib in the first-line setting. This phase III randomized study has three arms: encorafenib plus cetuximab; FOLFIRI or FOLFOX plus encorafenib and cetuximab; or the investigator's choice of standard chemotherapy with or without bevacizumab.

Different strategies are being investigated to improve the current results for this challenging population. New strategies to overcome resistances include immune checkpoint inhibitors or novel molecules such as ERK inhibitors or SHP2 inhibitors. Based on the immunogenic biological landscape of *BRAF* mutant mCRC, most current approaches included immune checkpoint inhibitors combinations. Trials have evaluated the combination of a BRAF inhibitor with a MEK inhibitor plus an immune checkpoint inhibitor. Results are available for two clinical trials. The first was a phase II trial evaluating the combination of dabrafenib–trametinib plus the anti-PD-1 spartalizumab,[48] giving an ORR of 33% and a DCR 76%. This trial included 21 patients regardless of their MSI status (4 MSS and 17 MSI). Among them, five patients had prior therapy with BRAF inhibitors and/or immunotherapy. The second is a phase I/II trial evaluating encorafenib plus cetuximab plus the anti-PD-1 nivolumab.[49] A total of 26 patients have been included, all of them are MSS; the ORR was 45%, DCR was 95%, with a median PFS of 7.3 months (95% CI, 5.6 to not reached), and median OS of 11.4 months (95% CI, 7.7 to not reached). An ongoing multiarm trial (NCT04294160), in previously treated (with or without previous BRAF inhibitors) *BRAF-V600E* mCRC patients, incorporates various combinations of the BRAF inhibitor dabrafenib with novel molecules: spartalizumab (anti-PD1), LTT462 (ERK inhibitor), TNO155 (SHP2 inhibitor), and LXH254 (BRAF/cRAF inhibitor). Further knowledge about mechanisms of resistance to target therapy will help to develop novel approaches to treat those patients. The role of BRAF inhibitors in terms of the detection of the *BRAF-V600E* mutation in ctDNA is being investigated in the adjuvant setting in the ACT-3 trial (NCT04259944). Patients who are ctDNA positive for the *BRAF-V600E* mutation after completion of 3–6 months of adjuvant treatment are considered as 'molecularly metastatic' and are randomized to surveillance or encorafenib plus binimetinib plus cetuximab.

Further research is also needed to identify the predictive biomarkers. In a small cohort of 23 patients treated with a *BRAF* inhibitor plus an anti-EGFR with or without a MEK inhibitor,

RNF43 somatic mutations were enriched in responders to BRAF inhibitor combination therapies, suggesting that differential activation of the WTN/B-catenin pathway might underlie differential sensitivity to BRAF inhibitors. All but one patient with a *BRAF* mutant tumor harboring *RNF43* achieved clinical benefit (CR, PR, or >6 months SD) with encorafenib plus cetuximab with or without binimetinib.[50] Regarding prognostic biomarkers, the *BRAF* mutant allele fraction in plasma was confirmed as a robust prognostic factor, regardless of the treatment.[50,51] Finally, previous bevacizumab treatment has also been suggested as a potential predictive biomarker. In the BEACON trial, OS among patients who received the triplet was lower among patients who had previously received bevacizumab compared with patients who did not receive previous anti-VEGF (HR, 1.74, 95% CI, 1.21–2.49). These results were not observed among patients who received encorafenib–cetuximab.[52]

Conclusions

In recent years, the therapeutic landscape of *BRAF-V600E* mCRC tumors has been completely reshaped, notably following the outcome of the BEACON trial. Prior to the development of BRAF inhibitors, standard chemotherapy has changed minimally giving only modest clinical outcomes. Subgroup analysis from several trials confirmed the benefit of adding an anti-VEGF drug; however, trials were not specific to the *BRAF-V600E* population. Despite the clinical improvement achieved with the combination of chemotherapy plus anti-VEGF, survival remains poor.

In stark contrast to the success observed in melanoma, BRAF inhibitors as monotherapy including vemurafenib, dabrafenib, and encorafenib have shown no activity in *BRAF-V600E* mCRC. Preclinical evidence pointed to rebound upregulation of EGFR as a critical component for successful BRAF inhibition. Dual blockade of both EGFR and BRAF resulted in synergistic inhibition of tumor growth in *BRAF-V600E* mutant CRC murine models, leading to new clinical combinations including an anti-EGFR, demonstrating more robust clinical activity. Subsequent strategies added a third MAPK pathway inhibitor, implementing MEK, ERK, or PI3CA blockade. The BEACON trial, which is the largest trial ever presented in the *BRAF-V600E* mCRC population, confirmed the benefit of the combination of encorafenib and cetuximab with or without binimetinib, over irinotecan-based chemotherapy. However, updated survival results demonstrated no differences in PFS or in OS for either the triplet or the doublet, despite the higher response rate among patients in the triplet arm. Based on this absence of differences but a better toxicity profile, both the FDA- and the EMA-approved encorafenib–cetuximab as a new standard of care for refractory *BRAF-V600E* mCRC. Of note, the higher response rate observed in the triplet arm of the BEACON trial was not associated with a PFS or OS improvement compared with the doublet. Interestingly, specific populations appear to benefit from triplet blockade; however, further prospective research is needed to identify the nature of these patient populations.

For upfront therapy, MSI status may help to guide treatment. Considering the results of the triplet in first line, the ANCHOR trial suggested that upfront targeted therapy was not active as expected for *BRAF-V600E*/MSS tumors. In an attempt to enhance the activity, the BREAKWATER trial is currently evaluating encorafenib–cetuximab combined with chemotherapy as upfront therapy. On the other hand, for patients with *BRAF-V600E*/MSI tumors, representing up to 30% of *BRAF* patients, the KEYNOTE-177 study demonstrated the outstanding effect of pembrolizumab as upfront therapy.[53] This benefit was further confirmed in the refractory setting with both pembrolizumab and nivolumab plus ipilimumab.[54,55] Therefore, pembrolizumab may be the first-line therapy choice for patients with *BRAF-V600E* mutant MSI CRC.

Despite the meaningful clinical activity observed in the BEACON trial and other trials evaluating BRAF inhibitors, not all patients responded and some responses are relatively short. This disparity in response highlights *BRAF-V600E* heterogeneity that has been confirmed through transcriptomic signatures. Given the immunogenic nature of *BRAF-V600E* mutant tumors, most are classified as CMS1 (MSI immune); studies have evaluated the combination of BRAF inhibitors plus immune checkpoint inhibitors with outstanding results, even among patients previously treated with either immunotherapy or BRAF inhibitors. Despite these important advances, *BRAF-V600E* mCRC remains a clinically challenging disease.[56–58] Current studies are evaluating several combinations with BRAF inhibitors such as

ERK1/2 inhibitors or SHP2 inhibitors that can overcome or delay acquired resistance. Considering these clinical advances in the *BRAF-V600E* field, future research should focus on identifying predictive and prognostic biomarkers as well as new strategies to overcome mechanisms of resistance.

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Not applicable.

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Author contributions

Javier Ros: Conceptualization; Writing – original draft; Writing – review & editing.

Nadia Saoudi: Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

Iosune Baraibar: Conceptualization; Writing – original draft; Writing – review & editing.

Francesc Salva: Writing – original draft; Writing – review & editing.

Josep Tabernero: Writing – review & editing.

Elena Elez: Writing – review & editing.

ORCID iD

Javier Ros https://orcid.org/0000-0002-8137-5415

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