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BRAF, *MEK* and *EGFR* inhibition as treatment strategies in *BRAF* V600E metastatic colorectal cancer

Javier Ros, Iosune Baraibar, Emilia Sardo, Nuria Mulet, Francesc Salvà, Guillem Argilés, Giulia Martini, Davide Ciardiello, José Luis Cuadra, Josep Tabernero and Elena Élez

Abstract

Introduction: *BRAF* driver mutations are found in up to 15% of patients with colorectal cancer (CRC) and lead to constitutive activation of *BRAF* kinase and sustained *RAS/RAF/MEK/ERK* pathway signaling. *BRAF* mutations define a sub-population characterized by a poor prognosis and dismal median survival. Following successful outcomes with *BRAF* inhibition in *BRAF* mutant metastatic melanoma, this approach was evaluated in metastatic colorectal cancer (mCRC). The development and combination of targeted therapies against multiple signaling pathways has proved particularly successful, with improved survival and response rates.

Areas covered: This review addresses the development of therapeutic strategies with inhibitors targeting MAPK/ERK and *EGFR* signaling in *BRAF* V600E mutated mCRC, focusing on encorafenib, binimetinib and cetuximab. A pharmacological and clinical review of these drugs and the therapeutic approaches behind their optimization are presented.

Expert opinion: Exploiting knowledge of the mechanisms of resistance to *BRAF* inhibitors has been crucial to developing effective therapeutic strategies in *BRAF-V600E* mutant mCRC. The BEACON trial is a successful example of this approach, using encorafenib and cetuximab with or without binimetinib in patients with previously treated *BRAF* V600E mutant mCRC, showing an impressive improvement in clinical outcomes and tolerable toxicity compared with chemotherapy, establishing a new standard of care in this setting.

Keywords: BEACON clinical trial, binimetinib, *BRAF* inhibitor, *BRAF* V600E mutation, colon cancer, *EGFR* inhibitor, encorafenib, *MEK* inhibitor

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Article highlights

- *BRAF-V600E* mutation in colorectal cancer (CRC) patients is associated with a poor prognosis and chemotherapy achieves only modest disease control.
- Therapeutic approaches with drugs targeting *BRAF-V600E* in CRC have not been as effective as in *BRAF* mutant melanoma, with *BRAF-V600E* inhibitor monotherapy giving an overall response rate of approximately 5%.
- Inhibition at a single step in the MAPK/ERK pathway with a *BRAF* inhibitor, results in adaptive feedback re-activation of MAPK signaling, often mediated by *EGFR* activation. Optimal pathway blockade is achieved by simultaneously targeting multiple steps of the pathway.
- Treatment with the *BRAF* inhibitor encorafenib and the anti-*EGFR*, cetuximab, with or without binimetinib, a *MEK* inhibitor, has shown impressive improvements in clinical outcomes in a context of tolerable toxicity, compared with standard chemotherapy.
- Understanding the mechanisms of resistance against *BRAF* inhibitor combinations is crucial for developing and optimizing new therapeutic strategies in metastatic colorectal cancer (mCRC), in order to identify patients most likely to obtain benefit from these targeted combinations.

Correspondence to:
Javier Ros
Department of Medical Oncology, Vall d'Hebron University Hospital, Passeig de la Vall d'Hebron, 119, Barcelona, Catalunya 08035, Spain
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
fjros@vhio.net

Iosune Baraibar
Francesc Salvà
Guillem Argilés
Elena Élez
Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Catalunya, Spain
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Josep Tabernero
Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, UVic-UCC, Passeig Vall d'Hebron, Barcelona, Spain

Emilia Sardo
Department of Medical Oncology, Vall d'Hebron University Hospital, Barcelona, Catalunya, Spain

Nuria Mulet
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain
Department of Medical Oncology, Institut Catala d' Oncologia, Barcelona, Spain

Giulia Martini
Davide Ciardiello
Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain

Medicine, Università degli Studi della Campania Luigi Vanvitelli, Naples, Caserta, Campania, Italy

José Luis Cuadra
IOB-Quiron, Barcelona, Spain

Introduction

Globally, colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women. Approximately two million new CRC cases were diagnosed worldwide in 2018 and the global burden is increasing every year, attributed in part to the adoption of western lifestyles. Numbers are expected to reach 2.2 million new cases and 1.1 million deaths by the year 2030.[1–3] Although cytotoxic chemotherapy with FOLFOX (5-fluorouracil, leucovorin and oxaliplatin) and FOLFIRI (5-fluorouracil, leucovorin and irinotecan) remains the backbone of care in metastatic colorectal cancer (mCRC), the identification of mutations driving tumorigenesis in CRC has considerably altered the therapeutic landscape over the past decade. The development of targeted therapies including anti-angiogenic agents targeting vascular endothelial growth factor (VEGF) signaling, *BRAF, MEK* and anti-epidermal growth factor receptor (*EGFR*) signaling, along with immunotherapy, and their combination with standard chemotherapy, has improved benefit in terms of both progression-free survival (PFS) and overall survival (OS) in specific mCRC subpopulations.[4–10]

The *RAS*/RAF/*MEK*/ERK pathway has also been implicated in the pathogenesis of mCRC. B-type *RAF* (*BRAF*) alterations are driver mutations that lead to constitutive activation of *BRAF* kinase and sustained *MAPK*/*ERK* signaling, resulting in increased cell proliferation and survival, and constituting a marker for dismal prognosis.[11] In this *BRAF-V600E*-mutated CRC population, aggressive triplet-based chemotherapy in combination with anti-VEGF therapies has achieved some success. Guided by outcomes in melanoma patients, targeted therapies have radically changed the therapeutic landscape for mCRC patients. The development of targeted inhibitors has considerably opened up therapeutic options for patients and clinicians. The main agents to show some degree of success include the anti-*EGFR* agent cetuximab, and later panitumumab, with more recent efforts focusing on alternative signaling pathways, notably *RAS*/RAF/*MEK*/ERK, with the development of da*brafe*nib, encorafenib and vemurafenib to block *BRAF*-mediated signaling, and trametinib and binimetinib to block *MEK* signaling.

We performed a review of PubMed and abstracts from major oncology congresses from January 2009 to June 2020 using MeSH and full-text search terms for molecular alterations in 'metastatic' or 'advanced' 'colorectal cancer/adenocarcinoma', as well as a range of therapy types, including *BRAF* and *MEK* inhibitors, anti-EGFR, anti-VEGF and chemotherapies in CRC. We provide an overview of the development of the main targeted therapies in *BRAF-V600E* mutated mCRC, with a focus on the pivotal and recent studies which define a role for the anti-*EGFR* monoclonal antibody cetuximab, the anti-*BRAF* inhibitor encorafenib, and the anti-*MEK* inhibitor binimetinib, as they take center stage in the treatment management of *BRAF*-V600E mutated CRC.

BRAF *pathway biology*

BRAF-V600E mutations are found in up to 20% of patients with CRC, with prevalence decreasing in the advanced setting.[12,13] The gene for *BRAF* kinase encodes a 766-amino acid serine/threonine protein that is involved in the mitogen-activated protein kinase (*MAPK*) pathway. This molecular pathway is composed of the cytoplasmic *RAS* proteins with GTPase activity (*H-RAS, K-RAS* and *N-RAS*), which recruit the *RAF* family proteins. On activation of *RAF* proteins, phosphorylation and activation of *MEK1/2* proteins occur, with subsequent phosphorylation and activation of *ERKs. ERKs* in turn phosphorylate a variety of substrates, including multiple transcription factors.[14] Sustained pathway activation results in increased cell proliferation and survival.

BRAF-V600E mutations occur in a wide range of cancer types and cause activation of downstream *MAPK*. They are mostly found in tumors in which *RAS* mutations are prevalent, such as CRC, lung cancer, malignant melanoma and borderline ovarian tumors, reflecting a setting in which the activation at any level of the *RAS*/RAF/*MEK*/ERK MAPK pathway may drive the cell towards carcinogenesis.[15] The missense activation mutation provokes the insertion of a negatively charged residue adjacent to the phosphorylation site within the catalytic domain that mimics phosphorylation and drives constitutive pathway activation.[16]

Approximately 20% of patients with *BRAF*-V600E-mutant mCRC present with microsatellite instability (MSI),[17,18] as somatic *BRAF*-V600E mutations increase *BRAF*/*MEK*/ERK signaling, resulting in the CpG island methylator phenotype and *MLH1* silencing through the transcriptional

repressor MAFG,[19,20] ultimately leading to deficient mismatch repair (dMMR). *BRAF*-mutant tumor subtypes based on gene expression have been described, clustering the population into two groups: BM1, defined by K*RAS*/AKT pathway activation, mTOR/4EBP deregulation, epithelial-to-mesenchymal transition and immune infiltration, and BM2, characterized by cell-cycle checkpoint dysregulation.[21]

Clinical-pathological features and treatment options in BRAF-*V600E mutant CRC*

The presence of a *BRAF-V600E* mutation is considered a marker for poor prognosis in patients with mCRC, associated with a median survival of approximately 12–14 months – before the introduction of targeted therapies, compared to 21–25 months for patients with *BRAF* wild-type (*BRAF* wt) tumors.[22,23] This mutation is associated with a particular phenotype and clinical, pathological and molecular characteristics. These include older age at diagnosis, female sex, and tumors located in the proximal colon with nodal and peritoneal spread. Pathologically, *BRAF-V600E* mutant CRC is associated with poorer differentiation, a mucinous histology, larger primary tumors, and K*RAS* wild type.[24,25] As commented previously, molecularly, *BRAF-V600E* is nearly always mutually exclusive with K*RAS* and approximately 20% of patients with *BRAF* V600E mutant mCRC are MSI-H.[17,26] Until recently, the combination of intensive chemotherapy with anti-VEGF therapies was considered the most appropriate approach for patients with *BRAF-V600E* mutated CRC, based on two phase III studies. The TRIBE trial was an open-label, randomized study in patients with unresectable mCRC, comparing bevacizumab combined with FOLFIRI or with FOLFOXIRI in the first-line setting. In a subgroup analysis of the 28 *BRAF-V600E* mutant patients, with the triplet chemotherapy proving more active than FOLFIRI plus bevacizumab (median OS was 19 and 10.7 months and median PFS was 7.5 and 5.5 months in the triplet and double combination, respectively).[27] Despite the benefit described in the TRIBE trial, recent data suggest that this approach does not confer benefit among these patients. Indeed, the TRIBE-2 trial evaluated the upfront exposure to the three cytotoxic drugs compared with a preplanned sequential strategy of doublets. The *BRAF* subgroup does not benefit from the intensive approach.[28] Furthermore, a recent individual patient data meta-analysis of FOLFOXIRI–bevacizumab *versus* doublets plus bevacizumab in previously untreated mCRC shows no increased benefit in terms of OS among this subgroup [hazard ratio (HR) 1.11; 95% confidence interval (CI) 0.75–1.73]. Thus, the use of FOLFOXIRI–bevacizumab should no longer be regarded as the first choice for patients with *BRAF*-V600E mutation, in whom the use of FOLFOX–bevacizumab seems to be the preferable upfront option.[29]

In the second-line setting, the VELOUR trial, a prospective randomized, double-blind study evaluated the efficacy and safety of aflibercept plus FOLFIRI *versus* placebo plus FOLFIRI in patients with mCRC, with disease progression on or after completing an oxaliplatin-based regimen. Analysis of the 36 *BRAF-V600E* mutant CRC patients showed an OS of 10.3 months with FOLFIRI aflibercept.[30] There have not been direct trials evaluating anti-VEGF specifically in *BRAF-V600E* mutant CRC which means that these results are from the subanalyses based on two clinical trials.

Regarding *EGFR*-targeted blockade in *BRAF-V600E* mutant mCRC, cetuximab was the first monoclonal antibody directed against *EGFR* to present preclinical efficacy.[31] It is a human/mouse chimeric recombinant IgG that binds to the extracellular domain of *EGFR* on both normal and tumor cells, competing with the endogenous *EGFR* ligand. On binding, cetuximab blocks receptor dimerization and phosphorylation, and is ultimately internalized and degraded. This translates into inhibition of cell growth, induction of apoptosis, and decreased matrix metalloproteinase and vascular endothelial factor production.

Following multiple clinical trials, cetuximab has been approved as first-line treatment in metastatic *KRAS* wild-type mCRC in combination with chemotherapy, and in later lines in patients refractory to irinotecan-based chemotherapy in combination with irinotecan, and as a single agent in patients who are chemorefractory or who are intolerant to irinotecan.[7,8,32–34] The main associated toxicities are skin reactions, notably in the form of acneiform rash (that may act as an early predictor of survival), severe hypomagnesemia and infusion reactions.[35]

However, the presence of *BRAF-V600E* mutations was found to be a negative predictor of response to anti-*EGFR* therapies in mCRC patients when combined with chemotherapy. A

subanalysis of patients with *BRAF-V600E*-mutant CRC from the phase III CRYSTAL trial evaluating the effect of the addition of cetuximab to FOLFIRI, showed that in the *BRAF-V600E*-mutant population the addition of cetuximab did not result in a significant benefit (median PFS 8.0 *versus* 5.6 months; HR = 0.934; $p = 0.87$, median OS 14.1 *versus* 10.3 months; HR = 0.908; $p = 0.74$).[36] Similar results were reported in a retrospective analysis of *BRAF-V600E*-mutant patients in the FIRE-3 study, in which patients were randomly assigned to either FOLFIRI plus cetuximab or FOLFIRI plus bevacizumab. While the objective response rate (ORR) was higher in the cetuximab arm compared to bevacizumab (52% *versus* 40%), results were comparable for median PFS (6.6 *versus* 6.6 months; HR = 0.84, $p = 0.56$) and OS (12.3 *versus* 13.7 months, HR = 0.79, $p = 0.45$),[37] again showing no benefit with the addition of cetuximab over anti-VEGF therapy. Thus, currently, anti-VEGF in combination with chemotherapy is recommended instead of chemotherapy plus anti-*EGFR* for *BRAF*-V600E mutated colorectal patients. Guidelines on the use of anti-*EGFR* currently mandate expanded *RAS*/*BRAF* testing and those patients with *BRAF*-V600E mutations should not be getting an anti-*EGFR* alone or in combination with chemotherapy.[38]

However, the control group in the BEACON clinical trial received either cetuximab and irinotecan or cetuximab and FOLFIRI (folinic acid, fluorouracil, and irinotecan) instead of anti-VEGF. That was consistent with European Society of Medical Oncology (ESMO) guidelines which recommend the use of cytotoxic doublet s containing 5-FU with and *EGFR* inhibitor in patients with mCRC which is *RAS* wild type whose disease has progressed on one prior regimen.[38]

Regarding the co-occurrence of *BRAF*-V600E and MSI/dMMR, In sporadic CRCs, the *BRAF* mutation is seen in approximately 60% of MSI high tumors and only 5–10% of microsatellite stable (MSS) tumors.[39,40] This is because the *BRAF-V600E* mutation results in hypermethylation of the MLH1 gene promoter, resulting in loss of the tumor suppressor function and leading to diminished DNA mismatch repair.[41] This occurs exclusively of the germline mismatch repair mutations seen in Lynch. Interestingly, MSI/*BRAF-V600E* mutant tumors could receive both target therapy and immunotherapy. Indeed, pembrolizumab has been agnostically approved by the US Food and Drug Administration (FDA) for patients with dMMR/MSI-High tumors. However, it is still unclear which the best therapeutic sequence is: immunotherapy then targeted therapy or target therapy then immunotherapy. Furthermore, in ASCO 2020, the results of the Keynote-177 study were presented.[42] This trial is an open-label phase III trial, comparing the programmed cell death protein 1 (PD-1) antibody pembrolizumab with standard-of-care chemotherapy as first-line treatment; PFS was the primary end-point. Patients receiving pembrolizumab had a median PFS of 16.5 months *versus* 8.2 months with chemotherapy (HR: 0.60; p=0.0002). Of note, *BRAF-V600E* mutated subgroups get benefit in terms of PFS (HR 0.48; 95% CI 0.27–0.86) whereas *KRAS* or *NRAS* mutated tumors do not get benefit (HR 1.19; 95% CI 0.68–2.07).

Non-V600E mutations in CRC. Non-V600E *BRAF* mutations occur in 2.2% of all mCRC cases, representing around 22% of all *BRAF* mutations detected by next-generation sequencing (NGS)-based testing. Those mutations exhibit a particular phenotype, different from the *BRAF*V600E mutation. In particular, non-V600E *BRAF* mutant patients are more often younger, men and have low-intermediate grade and left-sided primary tumors. Molecular differences are also apparent with non-V600E *BRAF* mutant patients, more likely to have concomitant RAS mutations than patients with *BRAF*-V600E mutations. Likewise, MSI is present at a lower frequency. Interestingly, most of these non-V600E *BRAF* mutations have impaired or missing kinase activity, such as *BRAF* D594 G or D594 N. Colorectal patients with non-V600E *BRAF* exhibit significantly longer median OS compared with the *BRAF* V600 mutant and also when compared with wild-type CRC (60.7 *versus* 11.4 *versus* 43.0 months, respectively). In fact, in multivariate analysis, non-V600E *BRAF* mutations were independently associated with improved OS (HR 0.18; $p = 0.001$).[43] Furthermore, non-V600 *BRAF* mutations do not have a negative impact on prognosis, and some preclinical studies suggest that sensitivity to *EGFR* inhibitors may be not decreased although it is important to note that *RAS* mutations are more frequent in this subgroup of *BRAF* mutant CRC.[44]

As not all *BRAF* mutations are activating and harbor a bad prognostic, Yao and colleagues classified the whole *BRAF*-activating mutations in preclinical functional models depending on their *RAS* signaling dependency and their conformational

functionality (monomer or dimer).[44,45] Three classes have been identified: class 1 includes the V600 mutations and *BRAF* protein acts as an active monomer; class 2 consists of kinase active mutations in codons 464, 469, 597 or 601 and *BRAF* acts as an active dimer; for both class (1 and 2) kinase activity is *RAS*-independent; class 3 mutations affect codons 287, 459, 466, 467, 469, 581, 594, 595 or 596, and *BRAF* can act as a dimer but has impaired his kinase activity, so signaling is *RAS*-dependent and is still sensitive to *ERK*-mediated inhibiting feedback.

Implementing *BRAF* and MAPK/ERK inhibition in *BRAF-V600E* mutant mCRC

Early clinical studies inhibiting EGFR *and MAPK/ERK signaling*

Preclinical studies investigated alternative therapeutic solutions for patients with *BRAF-V600E* mutant CRC. *In vitro* experiments suggested that resistance to monotherapy in *BRAF-V600E* mutant CRC cell lines after inhibition at a single step in the pathway resulted in increased *EGFR* phosphorylation and insensitivity to the *BRAF* inhibitor, could be mediated by adaptive feedback re-activation of MAPK signaling allowing sustained MAPK activation and continued cell proliferation.[11,46,47] In contrast, dual blockade of both *EGFR* and *BRAF* resulted in synergistic inhibition of tumor growth in *BRAF*-V600E mutant CRC murine models.[11]

Early clinical studies in *BRAF*-V600E mutant melanoma patients showed benefit with the *BRAF* inhibitor dabrafenib as a single agent or in combination with the *MEK* inhibitor trametinib, supporting the value of testing it in *BRAF*-V600E mutant mCRC patients. This approach was implemented along with simultaneous blockade of the *BRAF* and *EGFR* pathways using a combination of dabrafenib plus the anti-*EGFR* monoclonal antibody panitumumab with or without the *MEK* inhibitor trametinib.[48] Responses were achieved with dual *EGFR*/*BRAF* blockade in two out of 10 *BRAF-V600E* mutant mCRC patients (20%) with a median PFS of 3.4 months. Triple blockade with the addition of trametinib improved outcomes, with responses in nine 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 in the same study showed no responses and increased toxicity, notably skin toxicity.

In another trial, dual *BRAF* and *MEK* inhibition was evaluated with dabrafenib plus trametinib without an *EGFR* inhibitor in 43 patients.[49] Five patients (12%) achieved a response, including one complete response with a response lasting for 36 months. Interestingly, all nine on-treatment biopsies which were evaluable showed reduced levels of phosphorylated ERK relative to pretreatment biopsies. Furthermore, mutational analysis revealed that the patient achieving a complete response and two of three evaluable patients achieving a partial response had PIK3CA mutations suggesting that this mutation does not confers primary resistance to targeted therapies. Table 1 describes clinical outcomes with target therapy combinations and most relevant G3/4 adverse events.

Targeting *BRAF-V600E* mutant mCRC with *BRAF* inhibitors

Vemurafenib-based single agent and dual therapy strategies

The *BRAF* inhibitor vemurafenib (PLX4032) also had notable success in *BRAF*-V600E mutant melanoma. The first-in-man phase I study design evaluating the safety of single agent vemurafenib included two extension cohorts at the recommended phase II dose (RP2D) in mCRC and metastatic melanoma patients with a tumor harboring a *BRAF*-V600E mutation (NCT00405587). A total of 21 mCRC patients received vemurafenib [960 mg twice daily (BID)], and among the 19 patients evaluable for response, there was one partial response (PR) (5%) with a median PFS of 3.7 months.[50]

The basket trial of vemurafenib was notable as the first clinical trial which recruited based on a molecular alteration (*BRAF*-V600E mutation).[52] A total of 122 patients with *BRAF*-V600E mutant non-melanoma tumors received single agent vemurafenib. The CRC cohort was amended to include a vemurafenib–cetuximab combination due to insufficient activity with vemurafenib alone. However, the ORR for the combination was 4%, which was similar to that observed with vemurafenib monotherapy. When combined with the alternative anti-*EGFR* panitumumab two PRs were seen among the 12 (17%) evaluable patients. The combination was well tolerated with less cutaneous toxicity than expected with either single agent.[53]

Table 1. Clinical outcomes and G3/4 adverse events of targeted therapies in *BRAF* V600E mutant colorectal cancer.

Anti-*BRAF* drug regimen		Phase	Sample size	ORR (%)	PFS (months)	OS (months)	Main related AEs >G3 (%)	Reference
Monotherapy	Vemurafenib	I	21	5	3.7	NR	Rash and nausea 14%	Kopetz *et al.*[50]
	Encorafenib	I	18	0	4.0	NR	NR	Gomez-Roca *et al.*[51]
Two drug combination	Dabrafenib + trametinib	I/II	43	12	3.5	NR	Anemia 16%, pyrexia 12%, vomiting and fatigue 7%	Corcoran *et al.*[48]
	Vemurafenib + cetuximab	II	27	4	3.7	7.1	Lipase increased 22%, cutaneous squamous cell carcinoma 11%, abdominal pain 7%, arthralgia and diarrhea 4%	Hyman *et al.*[52]
	Vemurafenib + panitumumab	I	15	13	3.2	7.6	Alkaline phosphatase elevation 20%, fatigue and neutropenia 7%	Yaeger *et al.*[53]
	Dabrafenib-Panitumumab	I	20	10	3.5	13.2	Dry skin, hypomagnesemia and constipation 5%	Corcoran *et al.*[48]
	Encorafenib + cetuximab	III	220	20	4.2	8.4	Fatigue and anemia 4%, asthenia 3%, diarrhea and bilirrubin increased 2%	Kopetz *et al.*[54]
Triplet combination	Vemurafenib + cetuximab + irinotecan	Ib	18	35	7.7	NR	Diarrhea 5%, Leukopenia 4%, Arthralgia and anemia 2%	Hong *et al.*[55]
	Dabrafenib + trametinib + panitumumab	I	91	21	4.2	9.1	Rash 11%, dermatitis acneiform 10%, diarrhea and fatigue 7%, pyrexia 4%	Corcoran *et al.*[48]
	Encorafenib + cetuximab + alpelisib	I/IIb	54	18	4.2	NR	Dyspnea and hyperglycemia 11%, fatigue, nausea, hypophosphatemia, diarrhea, dermatitis acneiform and pyrexia 3.6%	van Geel *et al.*[56]
	Encorafenib + binimetinib + cetuximab	III	224	26	4.3	9	Diarrhea 10%, nausea 5%, acneiform dermatitis, fatigue, alanine aminotransferase and aspartate aminotransferase 2%	Kopetz *et al.*[54]

AEs, adverse events; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

In another phase Ib/II study, vemurafenib was evaluated at doses of 480 mg BID, 720 mg BID, and 960 mg BID, combined with cetuximab and irinotecan (also referred to as the VIC regimen) in 19 patients.[55] Of 17 evaluable patients, responses were observed in six (35%) patients, with a median Duration of Response (DOR) of 8.8 months and median PFS of 7.7 months. The most common adverse events (AEs) (≥30%) included fatigue (89%), diarrhea (84%), rash (74%), nausea (74%), anemia (74%), myalgia (53%), leukopenia (47%), arthralgia (42%), vomiting (32%), and neuropathy (32%). Interestingly, *BRAF*-V600E circulating cell-free DNA trends correlated with radiographic changes, and acquired mutations in genes reactivating MAPK signaling were observed at progression in these samples. This scheme is then included as a recommended regimen for *BRAF-V600E* mutant refractory CRC in the National Comprehensive Cancer Network (NCCN) guidelines.

Encorafenib: preclinical and clinical pharmacology

Encorafenib (LGX818) is a second generation *BRAF* inhibitor, developed subsequent to dabrafenib and trametinib. It is a highly selective adenosine triphosphate (ATP)-competitive small-molecule RAF kinase inhibitor which suppresses the *RAS*/RAF/*MEK*/ERK pathway in tumor cells expressing a *BRAF*-V600E mutation. Similar to other selective small-molecule *RAF* kinase inhibitors, encorafenib inhibits *CRAF* [half maximal inhibitory concentration (IC50) = 0.30 nM], *BRAF* (IC50 = 0.47 nM), as well as *BRAF*-V600E (IC50 = 0.35 nM) in cell-free assays. However, encorafenib does not inhibit *RAS*/RAF/*MEK*/*ERK* signaling in cells which do not harbor a *BRAF-V600E* mutation. In the human melanoma cell line A375, which expresses *BRAF*-V600E, encorafenib potently inhibits phospho-*MEK* [half maximal effective concentration (EC50) = 2 nM], phospho-*ERK* (EC50 = 3 nM), and proliferation (EC50 = 4 nM), resulting in cell cycle arrest and apoptosis.[57] With high selectivity compared to other kinases, encorafenib has no antineoplastic activity in tumor cell lines without a *BRAF-V600E* mutation and is highly selective for cell lines carrying the V600E/D/K *BRAF* mutations, with the greatest sensitivity observed in *BRAF*-V600E melanoma and CRC lineages.

Encorafenib is a relatively potent reversible inhibitor of cytochrome P450 (CYP)2B6, CYP2C9, and CYP3A4/5 and a weak (IC50 ⩾ 20 μM) reversible inhibitor of CYP1A2, CYP2C8, CYP2C19, and CYP2D6. It is also a potential inducer of CYP3A4. Metabolism occurs primarily *via* CYP3A4 (>50%) and to a lesser degree *via* CYP2D6 and CYP2C19. Furthermore, it inhibits uridine phosphate (UDP)-glucuronosyltransferase (UGT)1A1 and is a substrate of P-glycoprotein with high apparent passive permeability. In addition, encorafenib inhibits the renal transporters organic anion-transporting polypeptides (OAT)1, OAT3, and OCT2 and the hepatic transporters OATP1B1 and OATP1B3.

Encorafenib has been evaluated clinically in several tumor types, alone or in combination with other drugs, with early studies focusing on melanoma and CRC. It was initially evaluated in patients with locally advanced or metastatic *BRAF*-V600E melanoma in study CLGX818X2101, a phase I dose escalation and expansion study.[57] In cycle 1, encorafenib exposure on day 15 was consistently decreased by 30–60% compared with day 1, probably due to the induction of CYP450 enzymes. Area under the concentration-time curve (AUC) and maximum concentration (C_{max}) ratios at steady-state concentrations (day 15) relative to day 1 did not change with dose. The trough concentration on and after cycle 2 day 1 did not show a trend of further decline, suggesting that cycle 1 day 15 was close to or at the time of steady-state concentration. Two regimens were tested – up to 700 mg once daily (QD) and up to 150 mg BID. At these doses the average concentrations of encorafenib were above the predicted efficacious concentrations based on non-clinical xenograft models. Encorafenib was rapidly absorbed and detectable in plasma at 0.5 h post-dose and across all dose levels, peaking (T_{max}) at approximately 2 h. The terminal half-life (T1/2) was short (2.9–4.4 h), remained constant across doses, and was similar between day 1 and day 15. Seven melanoma patients experienced dose-limiting toxicities (DLTs), three of whom were treated at doses above 450 mg QD; the most frequent DLT was neuralgia (two patients, 4.1%). Encorafenib at a dose of 300 mg QD was declared the RP2D for evaluation in the expansion phase.

Encorafenib: monotherapy and dual BRAF *and* EGFR *inhibition in mCRC*

Encorafenib monotherapy was evaluated in patients with *BRAF*-V600E mutant refractory mCRC during the dose-expansion part of study CLGX818X2101.[51] A total of 18 patients (six at

300 mg QD; 12 at 450 mg QD) were treated. Antitumor activity was modest with an ORR of 5.6% and a disease control rate of 67%. Median PFS was 4.0 months. Three patients had DLTs of arthralgia and myalgia (one patient each), insomnia and myalgia (one patient), and bone pain and vomiting (one patient), all of which were grade 3 and all occurred with the 450 mg QD dose. The most common AEs of any grade were palmar-plantar erythrodysesthesia syndrome (67%), myalgia (44%), and dry skin (44%).

Given preclinical results supporting the value of the dual inhibition of *EGFR* and *BRAF*[11] along with clinical results of dual blockade with dabrafenib and panitumumab,[58] a phase Ib/II study was launched to evaluate encorafenib in combination with cetuximab and the phosphoinositide 3-kinase (PI3K) inhibitor alpelisib, because *in vitro* evidence suggests activation of the PI3K/AKT pathway is another possible mechanism of resistance to *BRAF* inhibitors.[59] The dose escalation part of the study evaluated encorafenib combined with cetuximab [400 mg/m² initial dose followed by weekly 250 mg/m² intravenously (IV)], either with (28 patients) or without (26 patients) alpelisib (a PI3Kα inhibitor).[56] Four encorafenib dose levels were evaluated (100 mg to 400 QD). Only patients treated with prior cetuximab or panitumumab were included in this dose-escalation part of the study; in the triple combination cohort, encorafenib 200 mg/alpelisib 300 mg and encorafenib 300 mg/alpelisib 200 mg in combination with the same cetuximab regimen. DLTs were reported in three patients receiving dual treatment (grade 3 arthralgia, grade 3 vomiting and grade 3 QT prolongation) and two patients receiving triple treatment (grade 4 acute renal failure and grade 3 bilateral interstitial pneumonitis); however, the MTD was not reached for either group. The RP2Ds selected were 200 mg QD encorafenib (both combinations) and 300 mg alpelisib. The most severe AEs were gastrointestinal, fatigue and hypophosphatemia, the toxicity profile was generally manageable. The ORR in the phase Ib part of this study was 19% in the 28 patients who received encorafenib plus cetuximab and 18% for patients who received triplet therapy with alpelisib. Median PFS was 3.7 and 4.2 months, respectively.

The phase II dose expansion part of the study enrolled 102 patients, 50 in the dual combination group and 52 in the triple combination group (encorafenib 200 mg QD + alpelisib 300 mg QD + cetuximab).[60] Patients with prior exposure to *EGFR*, PI3K, *MEK* or *RAF* inhibitors were excluded. Results were similar to those observed in the phase Ib part. A comparison of the triplet *versus* the doublet in terms of efficacy showed a HR (95% CI) of 0.69 (0.43–1.11; $p = 0.064$) with median PFS of 5.4 months (95% CI 4.1–7.2) and 4.2 months (95% CI 3.4–5.4), respectively, and an ORR of 27% (95% CI 16%–41%) and 22% (95% CI 12%–36%), respectively. That triplet combination achieves greatest clinical benefit.

Inhibiting MAPK/ERK signaling with a *MEK* inhibitor

Binimetinib: clinical pharmacology and monotherapy

Binimetinib (*MEK*162) is a novel *MAPK/ERK* pathway inhibitor, a non-ATP-competitive allosteric *MEK*1/2 that inhibits pERK in *BRAF-V600E*-mutant cancer cells. It is metabolized *via* multiple pathways, primarily by glucuronidation (mainly UGT1A1, 1A3 and 1A9) and to a lesser extent by oxidation (mainly CYP1A2 and 2C19). It has been investigated both as a single agent and in combination with *RAF* or *PI3K* inhibitors in advanced or metastatic solid tumors including melanoma, CRC, and biliary cancer.

Combing binimetinib with EGFR *inhibitors*

A combination of binimetinib with the anti-*EGFR* panitumumab was evaluated in patients with mCRC in the phase Ib/II study C*MEK*162X2116 (NCT01927341). During the dose escalation part, 10 patients were treated with binimetinib at a dose of 45 mg BID and panitumumab (6 mg/kg IV BID). Forty patients were enrolled in the phase II part (same doses), and the most common AEs regardless of causality, including diarrhea (70% all grades; 13% grade 3–4), vomiting (55%/2.5%), rash (50%/13%), nausea (48%/5.0%), fatigue (35%/5.0%), abdominal pain (33%/2.5%), dermatitis acneiform (33%/5.0%), blood creatine kinase increased (28%/7.5%), hypokalemia (20%/13%), AST increased (18%/5.0), blood creatinine increased (15%/2.5%), and hypomagnesemia (15%/0%).

The combination of binimetinib with encorafenib as dual or triple combination therapy was investigated in three clinical studies in patient with a range of tumor types harboring a *BRAF*-V600 mutation; the C*MEK*162X2110[61] trial provides

dosing and safety data. The first of these trials was an open-label, dose-finding, phase Ib/II study to determine the MTD and RP2D of binimetinib in combination with encorafenib (dual combination), and in combination with encorafenib and LEE011 (triple combination), in selected patient populations (locally advanced or metastatic melanoma, mCRC or any other solid tumor, all positive for a *BRAF*-V600E mutation).[61] In the phase Ib part, 47 patients were treated with binimetinib 45 mg BID and encorafenib at seven dose levels from 50 to 800 mg QD. The MTD was not reached (highest tested dose was 45 mg + 800 mg, respectively). Initially, two RP2Ds were declared for the combinations 45 mg + 450 mg and 45 mg + 600 mg dose levels. Among the 79 patients treated with the dual combination in the phase II part, 15 received encorafenib at 400 or 450 mg QD and 64 were treated with ⩾ 600 mg QD. The most common AEs (⩾20%) were diarrhea, nausea, vomiting, arthralgia, fatigue, pyrexia, constipation, AST increased, blood creatine kinase (CK) increased, ALT increased, retinopathy, and cough. Regardless of causality, the most common grade 3 or 4 AEs (⩾3.0%) were increases in serum lipase, liver enzymes (ALT, AST), and creatine kinase, diarrhea, nausea, vomiting, and anemia. Interestingly, compared to the respective single-agent therapies, there was a decreased occurrence of skin toxicities with the combination.

The BEACON study: dual and triple blockade of EGFR *and MAPK signaling in mCRC*

The practice-changing phase III BEACON trial evaluated targeted therapy for dual and triple targeted blockade in refractory *BRAF* V600E CRC. Patients were randomly assigned (1:1:1) to receive the triple combination of encorafenib plus cetuximab and binimetinib, the encorafenib plus cetuximab doublet, or irinotecan-based chemotherapy plus cetuximab.[54] Median OS was 9.0 months (95% CI 8.0–11.4) for the triplet targeted therapy compared to 5.4 months (95% CI 4.8–6.6) for standard chemotherapy-based treatment (HR 0.52; 95% CI 0.39–0.7; $p < 0.0001$). Median OS for the doublet combination was 8.4 months (95% CI 7.5–11.0) compared to standard therapy (HR 0.6; 95% CI 0.45–0.79; $p < 0.0003$). Median PFS was 4.2, 4.1 and 1.5 months for the triplet, the doublet combination and chemotherapy, respectively. Unfortunately, the study was not powered to compare the triplet and doublet therapies. The confirmed ORR for the triplet targeted therapy was 26% (95% CI 18–35) compared to 2% (95% CI 0–7; $p < 0.0001$) for standard therapy. The toxicity profile revealed that treatment was globally well tolerated, with grade 3 or higher AEs in 58% of patients on triplet treatment, 50% in the doublet group and 61% with standard therapy. The trial used four validated patient-reported outcome measurement tools: the European Organisation for Research and Treatment of Cancer QOL questionnaire, Functional Assessment of Cancer Therapy, EuroQol 5D 5L, and the Patient Global Impression of Change. Patients treated with the triplet had an approximately 44–45% reduction in quality of life deterioration compared with patients in the standard of care group, based on the quality of live tools. Those receiving the doublet had an approximately 46% reduction in risk. These results led to approval in May 2020 of the doublet combination (not the triplet because of the comparable clinical outcomes) encorafenib and cetuximab for adults with mCRC whose tumors have the *BRAF-V600E* mutation, and who have already undergone at least one prior treatment regimen.

Despite the impressive results of the BEACON clinical trial, not all patients respond to this therapeutic approach and some of the responses are short. This disparity in response highlights *BRAF-V600E* mutant CRC heterogeneity. Indeed, some authors have suggested that transcriptome can partially explain *BRAF-V600E* heterogeneity and *EGFR*/*BRAF*/*MEK* inhibitor efficacy. Barras *et al.* distinguished two subtypes of V600E *BRAF* mutants according to the gene expression profile: BM1 and BM2.[21] BM1 represents 30% of all *BRAF-V600E* mutant CRC tumors and is characterized by K*RAS*/AKT pathway activation, *mTOR*/*4EBP1* deregulation and epithelial–mesenchymal transition (EMT). BM2 represents almost 70% of all *BRAF-V600E* mutant CRC tumors and is characterized by cell-cycle and cycle checkpoints-related deregulation. On the other hand, BM1 exhibits a stronger immune profile (*IL2*/*STAT5*/*IL6*/*JAK*/*STAT3* pathway activation, enrichment in angiogenesis, TNF-alfa signaling and allograft rejection). BM2 tumors are enriched in metabolic processes and display high CDK1 and low cyclin-D1 levels. Interestingly, BM classification is independent of MSI status, methylation patterns, *PI3K* mutational status, sidedness and gender. BM1 exhibits poorer prognosis compared to BM2 subtypes; thus suggesting that the *BRAF*-V600E mutation does not confer a unique biology and providing a

deep characterization that could be exploited for drug targeting.

The preclinical data and the encouraging preliminary efficacy results observed in the safety lead-in (SLI) part of the BEACON trial justify the evaluation of encorafenib, binimetinib and cetuximab in the first-line setting of this subject population. This triplet therapeutic strategy is currently being explored as a frontline approach in the *BRAF* V600E mutant mCRC population in the ongoing phase II single-arm ANCHOR-CRC trial, and results are expected by the end of 2020 (NCT03693170). This trial is a phase II, single-arm study, evaluating the triple combination for previously untreated *BRAF-V600E* mutated CRC. The results of stage 1 were presented at the World GI Congress 2020.[62] Forty patients were enrolled. The primary endpoint was ORR assessed *via* local review, and secondary endpoints included PFS and safety. Population characteristics included a median age of 67 years (36–80), up to 70% of women, 68% of right-sided tumors and 78% of patients with two or more metastatic organs. The confirmed response rate was 50%, with a disease control rate of 85% (50% partial response, 35% stable disease). Median PFS was 4.9 months (95% CI 4.4–8.1). Regarding toxicity, the triple combination was well tolerated and manageable with no unexpected toxicities (grade ⩾3: 68%). Most frequent adverse events were comparable to those observed with the same triplet combination in the BEACON study. Having reached the minimal number of confirmed responses in stage 1, the futility analysis allowed us to enroll additional patients in stage 2. The trial is currently ongoing.

Conclusion

CRC is a notably heterogeneous disease. A better understanding of the molecular mechanisms of carcinogenesis has allowed improvements in the management of this disease and the expansion of new therapeutic strategies. *BRAF-V600E* mutations have been observed in between 8% and 15% of patients with mCRC.[12,13] The most common of these mutations is *BRAF*-V600E, and it is bestowed with a notably worse prognosis, along with a particular phenotype and clinical and pathological characteristics.

Before the era of *BRAF* inhibitor combinations, the combination of intensive chemotherapy with anti-VEGF therapies was considered the most appropriate approach not only in first-line but also in second-line treatment for patients with mCRC harboring a *BRAF-V600E* mutation. In this setting, both the TRIBE clinical trial (FOLFOXIRI-bevacizumab) and the VELOUR clinical trial (FOLFIRI-aflibercept) showed an improvement in OS.

At a molecular level, *BRAF-V600E* mutations in CRC are known to be nearly always mutually exclusive with *KRAS* and activate downstream *MAPK* regardless of *RAS* status, explaining why the inhibition of *BRAF* with a single agent (and hence of a single step of the pathway) such as vemurafenib and dabrafenib, has not demonstrated therapeutic benefits, unlike in the setting of *BRAF* mutant melanoma. Learning once again from the melanoma experience, several studies with different agents and combinations were performed in an attempt to evaluate the optimal combination to improve clinical outcomes in mCRC. The phase III BEACON trial changed clinical practice following the demonstration that both the dual therapy (encorafenib + cetuximab) and the triple combination (encorafenib + cetuximab + binimetinib) increase OS, PFS and ORR compared to standard therapy of chemotherapy with cetuximab.

Thanks to this change of scenario, we have seen the advent of a new era in *BRAF*-V600E-mutated mCRC, making available not only standard treatment but also targeted therapies with effective results. Taking the safety profile into consideration is important, given that the rate of grade 3 or higher AEs is 50% and 58% for the double and triple combinations, respectively, highlighting the critical aspect of correct patient selection when choosing a combination therapy.

Expert opinion

The presence of a *BRAF*-V600E mutation in CRC portends a very poor prognosis. Typically, survival is about half as long as that of *BRAF* wild-type patients, reflecting the critical need to find new treatments that meaningfully change clinical outcomes of *BRAF* mutant CRC patients. The last decade has seen extensive efforts put into identifying efficient treatments, particularly with respect to MAPK pathway blockade. Several studies revealed very disappointingly that patients with *BRAF*-V600E mutated CRC generally do not respond to *BRAF* inhibitors in the same way as patients with *BRAF*-mutated melanoma. Response rates with single agent

BRAF inhibitors achieve only anecdotal responses. Fortunately, the BEACON trial demonstrated clearly that both the double and the triple targeted therapy combinations improve clinical outcomes compared with standard chemotherapy in terms of ORR, PFS and OS, in refractory mCRC patients harboring a *BRAF* mutation. Outcomes are also better than the highly intensive regimens of chemotherapy such as FOLFOXIRI plus bevacizumab.

Most patients with refractory *BRAF*-V600E mutated CRC will obtain benefit with this *MAPK* targeted multiple blockade approach. Nonetheless, not all patients respond to the treatment and some responses are short. Developing predictive biomarkers better to identify those patients who will achieve greatest benefit remains an urgent necessity. We also need more accurate prognostic factors that could contribute to more accurate clinical trial designs. Furthermore, despite these impressive improvements, identifying which combination is better, the triplet or doublet, remains unknown, as the BEACON trial was not designed to compare them directly. Nonetheless, indirect comparisons suggest that both experimental arms could be equivalent, without relevant differences in clinical outcomes. Regarding toxicity, the toxicity profile was acceptable, with grade 3 or higher AEs seen in 58% of patients on triplet treatment, 50% in the doublet group and 61% in the standard therapy group. There were not significant differences in terms of quality of life between the triplet and the doublet combination. There was no significant difference in quality of life for patients in the triplet and doublet groups, highlighting that with these novel targeted therapy regimens, not only is disease controlled for longer, but patient-reported quality of life is maintained for longer.

Taking these clinical outcomes together, using dual and triple combinations to block multiple signaling pathways offers a clear improvement on previous options, and suggest that a maximum of patients should receive the doublet or triplet encorafenib plus binimetinib- based regimens to achieve the greatest benefit with a minimal impact on their quality of life.

However, the best sequence strategy, chemotherapy *versus* target therapy, is still debated. In the ANCHOR trial the triple combination showed an ORR of 50% (95% CI 33.8–66.2) with 85% of patients having a decrease in tumor size.[62] Nevertheless, despite an initial response, most of the patients rapidly progress to the treatment (PFS was 4.9 months, 95% CI 4.4–8.1). Similar data were reported for the combination of doublet or triplet chemotherapy.[27,63] In a subgroup analysis of *BRAF-V600E* mutant patients in the TRIBE study, PFS was 7.5 months, ORRs of 56% were reported for patients receiving FOLFOXIRI + bevacizumab.

Comparable results were recently observed for the combination of FOLFOXIRI + panitumumab in the VOLFI trial.[63] In order to define the best treatment strategy for *BRAF-V600E* mutant CRC, a phase III study is planned. In the BREAKWATER trial 870 patients with untreated *BRAF* V600E MSS mCRC will receive encorafenib and cetuximab plus FOLFOX/FOLFIRI or a physician's choice represented by a chemotherapy doublet or triplet ± bevacizumab.

Another promising novel therapeutic option is represented by the combination of target therapy with immunotherapy. At the WGI congress 2020, Corcoran and colleagues presented the preliminary results of a small phase II study evaluating the association of dual *BRAF* and *MEK* inhibition, respectively, with dabrafenib and trametinib, with the anti-PD-1 spartalizumab.[64] Interestingly, the ORR was 35% (7/20) and disease control rate of 75%, which compares favorably with the historical 12% ORR of dabrafenib plus trametinib.[49]

Moreover nine out of 20 patients remained on therapy for more than 6 months. Serial ctDNA analysis displayed a decrease in *BRAF-V600E* ctDNA levels in responders and the emergence of MAPK pathway alterations on acquired resistance. Single-cell RNAseq showed an increase in infiltration by T-cells and other immune populations after the first cycle of treatment, as well as increased expression of genes correlated with T-cell cytotoxic activity.

The follow-up question is that of the optimal treatment sequence: targeted therapy followed by chemotherapy plus anti-VEGF or chemotherapy plus anti-VEGF followed by targeted therapy. Liquid biopsies and tumor samples at time of tumor progression are one means of allowing us to understand the mechanism of resistance against these targeted agents and determine more accurate subsequent treatments. Indeed, several trials confirm that cfDNA and *BRAF* mutant allele fraction predict and mirror radiographic response and could allow identification of new mechanisms of acquired resistance.[49,55]

Author note

Javier Ros is also affiliated with Università degli Studi della Campania Luigi Vanvitelli, Naples, Caserta, Campania, Italy.
Josep Tabernero is also affiliated with Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain.

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