Gastroenterology Report, 8(3), 2020, 192–205

doi: 10.1093/gastro/goaa022
Advance Access Publication Date: 15 June 2020
Review

REVIEW

BRAF and *KRAS* mutations in metastatic colorectal cancer: future perspectives for personalized therapy

Zi-Nan Li[1,2,†], Lin Zhao[1,2,†], Li-Feng Yu[1,2] and Min-Jie Wei[1,2,*]

[1]Department of Pharmacology, School of Pharmacy, China Medical University, Shenyang, Liaoning, P. R. China; [2]Liaoning Engineering Technology Research Center, China Medical University, Shenyang, Liaoning, P. R. China

*Corresponding author. Department of Pharmacology, School of Pharmacy, China Medical University, No. 77 Puhe Road, Shenyang North New Area, Shenyang, Liaoning 110122, P. R. China. Tel: +86-13358866401; Email: mjwei@mail.cmu.edu.cn

†These authors contributed equally to this work.

Abstract

Colorectal cancer (CRC) is one of the most commonly diagnosed cancers worldwide and 30% of patients with CRC experience metastasis. Patients with metastatic colorectal cancer (mCRC) have a 5-year overall survival rate of <10%. V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) and V-Ki-ras2 Kirsten ratsarcoma viral oncogene homolog (*KRAS*) mutations are mostly studied in mCRC, as clinical trials found that first-line chemotherapy with anti-epidermal growth factor receptor agent confers limited efficacy for mCRC. Treatment decisions for early-stage mCRC do not consider *BRAF* or *KRAS* mutations, given the dramatically poor prognosis conferred by these mutations in clinical trials. Thus, it is necessary to identify patients with mCRC harboring *BRAF* or *KRAS* mutations to formulate rational therapeutic strategies to improve prognosis and survival. *BRAF* and *KRAS* mutations occur in ∼10% and ∼44% of patients with mCRC, respectively. Although the survival rate of patients with mCRC has improved in recent years, the response and prognosis of patients with the aforementioned mutations are still poor. There is a substantial unmet need for prospective personalized therapies for patients with *BRAF*- or *KRAS*-mutant mCRC. In this review, we focus on *BRAF* and *KRAS* mutations to understand the mechanisms underlying resistance and improving the response rate, outcomes, and prognosis of patients with mCRC bearing these mutations and to discuss prospective personalized therapies for *BRAF*- and *KRAS*-mutant mCRC.

Key words: metastatic colorectal cancer; epidermal growth factor receptor; *KRAS* mutation; *BRAF* mutation; personalized therapy

Introduction

Colorectal cancer (CRC) is the third most commonly occurring cancer in men and the second most common cancer in women. More than 1.8 million new cases were diagnosed in 2018. Approximately 20% of new colorectal cancer cases are metastatic at the time of diagnosis and another 20% of cases develop into metastatic colorectal cancer (mCRC), which has a significantly lower survival rate [1, 2]. In recent decades, monoclonal antibodies (mAbs) targeting vascular endothelial growth factor (VEGF) and the epidermal growth factor receptor (EGFR) have been used as the first-line treatments for mCRC [3, 4]. Studies indicate that, in the first-line treatment setting, addition of an anti-VEGF antibody therapy improves the median overall survival (OS) [5]. However, unlike VEGF inhibitors, the efficacy of anti-EGFR agents, such as cetuximab and panitumumab, is limited to patients with wild-type (wt) V-Ki-ras2 Kirsten rat

Submitted: 30 December 2019; **Revised:** 2 March 2020; **Accepted:** 9 April 2020

sarcoma viral oncogene homolog (*KRAS*) tumors without the V-raf murine sarcoma viral oncogene homolog B1 (*BRAF*) mutation. Given the poor prognosis of *BRAF*- and *KRAS*-mutant mCRC, here, we focus on the *BRAF* and *KRAS* mutations, which exhibit two consensus subtypes.

KRAS, *NRAS*, and *HRAS* are some of the most well-studied rat sarcoma virus (RAS) subfamily proteins because of their significant role in cancer [6]. Of these *RAS* mutations, *KRAS* mutations (85%) are observed most frequently, followed by *NRAS* (15%) and *HRAS* (1%). *KRAS* mutations occur in approximately 44% of mCRC, with the majority observed in codons 12 and 13 of exon 2 (80% are G12D, G12V, G12C, G12A, and G13D) and less commonly in codon 61 of exon 3 (5% are Q61H, Q61L, and Q61R) and codon 146 of exon 4 (2% are A146T and A146V) [7]. Analyses of clinical-trial data strongly suggest that *KRAS* codon 12 or 13 mutations are a major predictive biomarker for resistance to anti-EGFR therapy in patients with mCRC [8–11]. These sites play a part in the activation of v-Raf murine sarcoma viral oncogene (RAF) kinases and mutations in these sites cause constitutive KRAS activation (without ligand-receptor binding), producing resistance to anti-EGFR agents [11, 12].

NRAS, the RAS homolog, has identical effector binding domains to *KRAS*. NRAS protein is part of the mitogen-activated protein kinase (MAPK) cascade [13, 14]. Membrane-bound NRAS proteins (189 amino acids) have GTPase activity and play a role in cell-signal transduction. Mutations in *NRAS* may result in structural activation and increased affinity for downstream effectors of the MAPK-signaling pathway—a key event in many cancers. Hence, *NRAS* mutations in codons 12, 13, 61, and 146 yield similar effects to *KRAS* activation [15–17]. *NRAS* mutations also have predictive value in anti-EGFR treatment resistance. Randomized clinical trials have shown that *NRAS* mutations in exons 2, 3, and 4 can be predictive markers of a lack of clinical benefit of anti-EGFR treatment when given in combination with chemotherapy in the first-line setting [14–16]. Patients bearing wt-*RAS* CRC treated with anti-EGFR therapy showed higher response rates and OS than those with *RAS* mutations (*KRAS* and *NRAS* exons 2, 3, and 4) [18–20]. Patients with mCRC with mutations in *NRAS* exons 2, 3, and 4 showed a poor overall response rate (ORR) and an adverse effect on progression-free survival (PFS) and OS [18, 21, 22]. In another study, patients with *NRAS* mutations developed more distant metastases and the 3-year risk in patients with *NRAS* mutations was 40.0% compared with 12.2% in patients with wt *NRAS* [23], indicating the significance of *NRAS* mutations in patients with CRC.

BRAF belongs to the RAF family of kinases, which also includes *ARAF* and *CRAF* [24]. *BRAF* mutations are less frequent than *KRAS* mutations in mCRC: ~10% of patients with mCRC bear mutations in *BRAF*. A single substitution missense mutation in V600 comprises approximately 90% of these *BRAF* mutations and nearly 90% of V600 mutations involve substitution to glutamic acid (V600E) [25, 26]. V600 is necessary for *BRAF* to remain inactive in the absence of an activation signal from RAS. The V600E substitution results in constitutive MAPK phosphorylation and subsequent RAF-mitogen-activated protein kinase (MEK)-extracellular signal-regulated kinase (ERK) signal transduction [27–29]. *BRAF* mutations are associated with poor prognosis in mCRC and mortality is nearly triple that of patients with wt-*BRAF* [30, 31].

Over the past two decades, the molecular characterization of mCRC has been revolutionized by the implementation of routine *KRAS*- and *BRAF*-mutation testing. Reportedly, *BRAF* and *KRAS* mutations are associated with a very poor prognosis. In terms of treatment, patients with these mutations show poor response to anti-EGFR treatment [32, 33]. Studies analysing the *KRAS*- and *BRAF*-mutation status of patients with mCRC found that patients with these mutations had lower PFS and OS rates than those with wt-*KRAS* and *BRAF*. Therefore, identification of *BRAF* or *KRAS* mutations can play an important role in improving the response rate and survival in mCRC treatment.

Although, with the advancement in systemic chemotherapy, targeted therapies, and surgical techniques, the survival of patients with mCRC has been improved in the past two decades, a few patients show poor response to treatment and continue to have a poor prognosis; particularly, patients with *BRAF* and *KRAS* mutations show a poor response and inferior survival. Thus, this review will focus on recent advances in personalized therapies for *BRAF*- and *KRAS*-mutant mCRC. Here, we discuss the mechanisms of *BRAF* and *KRAS* mutations that cause resistance to targeted therapies and propose future perspectives for personalized therapy for these patients.

KRAS and *BRAF* mutations as prognostic and predictive biomarkers in mCRC treatment

mAbs that target EGFR can achieve antitumor efficacy and are used as part of the standard treatment for mCRC. However, in clinical trials comparing therapeutic strategies plus anti-EGFR agents cetuximab or panitumumab, the outcomes are not satisfactory [5]. As shown in Table 1, PFS and OS were reduced in patients treated with an anti-EGFR agent compared with those in patients treated without this agent. In the CAIRO2 trial, patients received either capecitabine–bevacizumab (CB) or capecitabine–bevacizumab–cetuximab (CBC). The addition of cetuximab significantly decreased the median PFS and OS [34]. The PACCE study, a phase III trial, evaluated the efficacy of oxaliplatin–bevacizumab (OB) and irinotecan–bevacizumab (IB), with or without panitumumab. Both PFS and OS improved following OB/IB treatment than following OBP/IBP treatment [35]. Both these phase III studies (CAIRO2 and PACCE) indicate that the addition of an anti-EGFR antibody resulted in an inferior outcome.

Given these unexpected results and the results of studies at the preclinical and clinical levels, which suggest that the presence of mutated *KRAS* or *BRAF* is a predictive marker of anti-EGFR resistance [29, 36–45], we analysed the *KRAS*- and *BRAF*-mutant status and corresponding PFS and OS of each treatment group across several trials (Table 2). In these trials, the PFS and OS are lower in patients with *KRAS* and *BRAF* mutations than in patients with wt-*KRAS* and *BRAF* when undergoing anti-EGFR treatment. [34, 35, 46–48]. The effects of treatment without an anti-EGFR agent are greater than those with an anti-EGFR agent in patients with *KRAS* and *BRAF* mutations. In addition, one study in particular reported that, out of 79 patients with CRC, 11 had *BRAF*

Table 1. Effect of treatment with anti-EGFR agents for patients with mCRC harboring *BRAF* or *KRAS* mutations

Trial	Treatment	PFS (95% CI, months)	OS (95% CI, months)
CAIRO2	CB	10.7 (9.7–12.3)	20.3 (17.8–24.7)
	CBC	9.4 (8.4–10.5)	19.4 (17.5–21.4)
PACCE	OB	11.4 (10.5–11.9)	24.5 (20.4–24.5)
	OBP	10.0 (8.9–11.0)	19.4 (18.4–20.8)
	IB	11.7 (9.0–13.2)	20.5 (19.8 to NE)
	IBP	10.1 (8.2–13.7)	20.7 (17.8 to NE)

CB, capecitabine–bevacizumab; CBC, capecitabine–bevacizumab–cetuximab; OB, oxaliplatin-based chemotherapy and bevacizumab; OBP, oxaliplatin-based chemotherapy, bevacizumab, and panitumumab; IB, irinotecan-based chemotherapy and bevacizumab; IBP, irinotecan-based chemotherapy, bevacizumab, and panitumumab; NE, not estimable.

Table 2. Clinical trials of the effect of treatment with anti-EGFR agents for patients with mCRC harboring *BRAF* or *KRAS* mutations

Name of study	Mutated gene type	Phase of clinical trial	No. of patients	Therapeutic strategy	Patients with *KRAS*/*BRAF* mutations		PFS (months)				OS (months)			
							Wild-type		Mutant		Wild-type		Mutant	
					No.	%	With anti-EGFR	Without anti-EGFR	With anti-EGFR	Without anti-EGFR	With anti-EGFR	Without anti-EGFR	With anti-EGFR	Without anti-EGFR
PACCE	*KRAS*	III	664	OB/OBP	260	39	9.8	11.5	10.4	11.0	20.7	24.5	19.3	19.3
			201	IB/IBP	86	43	10.0	12.5	8.3	11.9	NE	19.8	17.8	20.5
CAIRO2		III	520	CB/CBC	206	40	10.5	10.6	8.1	12.5	21.8	22.4	17.2	24.9
CRYSTAL		III	1,063	FOLFIRI, cetuximab	397	37	9.9	8.4	7.4	7.7	23.5	20.0	16.2	16.7
PRIME		III	1,096	FOLFOX4, panitumumab	440	40	9.6	8.0	7.3	8.8	23.9	19.7	15.5	19.3
CRYSTAL	*BRAF*	III	519	FOLFIRI, cetuximab	45	8.7	10.4	12.2	6.6	5.9	21.5	24.6	15.2	15.0
TAILOR		III	393	FOLFOX4, cetuximab	52	13	9.3	7.5	2.0	3.8	–	–	–	–

NE, not estimable; OB, oxaliplatin-based chemotherapy, bevacizumab; OBP, oxaliplatin-based chemotherapy, bevacizumab, panitumumab; IB, irinotecan-based chemotherapy, bevacizumab; IBP, irinotecan-based chemotherapy, bevacizumab, panitumumab; CB, capecitabine, oxaliplatin, bevacizumab; CBC, capecitabine, oxaliplatin, bevacizumab, cetuximab; FOLFIRI, irinotecan, infusional fluorouracil, leucovorin; FOLFOX4, infusional fluorouracil, leucovorin, and oxaliplatin.

mutations and none of these 11 patients responded to anti-EGFR treatment [37].

Several clinical trials demonstrated that mutated *KRAS* and *BRAF* genes are predictive markers of outcomes in mCRC treatment (Table 3). In addition to the trials discussed above, the table includes several trials that indicate the predictive effect of *BRAF* and *KRAS* mutations [18, 31, 34, 46, 48–52]. These trials clearly demonstrated that the mutation statuses of *BRAF* and *KRAS* were predictors of outcome for mCRC patients when undergoing anti-EGFR treatment. Patients harboring *BRAF* or *KRAS* mutations showed poor prognosis and lower survival in mCRC.

Based on the clinical-trial data shown in Figure 1 [37], we can conclude that patients with mCRC bearing *BRAF* and *KRAS* mutations have poor prognosis with anti-EGFR therapies, which make *KRAS* and *BRAF* mutations prognostic and predictive biomarkers in anti-EGFR combined mCRC-treatment regimens [37]. In addition, some studies report that the *BRAF* mutation and the *RAS* mutation are mutually exclusive in mCRC [29, 53, 54]. None of the patients bearing the *KRAS* mutation carried the associated *BRAF* mutation in these trials. In other words, the *BRAF* mutation occurred exclusively in wt-*KRAS* mCRC.

The mechanism of anti-EGFR-therapy resistance in *BRAF*- and *KRAS*-mutant mCRC

KRAS, *NRAS*, and *BRAF* are the most-studied mutant genes in mCRC and current data indicate that mutation status is associated with resistance to single-agent cetuximab or panitumumab in patients with mCRC [21, 55]. Randomized studies have shown that patients with mutations in *KRAS* exons 3 and 4 or *NRAS* exons 2, 3, and 4 showed decreased response rates and OS when therapy was combined with an anti-EGFR treatment [18–20]. The *BRAF* mutation is also a strong indicator of poor prognosis in mCRC; the addition of an anti-EGFR agent had a detrimental effect on survival in *BRAF*-mutant patients with mCRC [18, 56, 57]. Only 10% of patients with chemotherapy–refractory mCRC achieve a positive response to anti-EGFR agents as a single agent [58, 59]. It seems that molecular alterations in the nodes of the EGFR-signaling pathway contribute to primary resistance to anti-EGFR treatment. Given that gene-expression signatures that correspond to *KRAS*, *BRAF*, and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*)-activating mutations have predictive value on the efficacy of anti-EGFR therapy, downstream components of *KRAS*, *BRAF*, and *PIK3CA* play important roles in anti-EGFR resistance [60–62].

Normally, EGF selectively binds to EGFR and triggers the receptor to form a dimer that activates receptor tyrosine kinase (RTK) activity and RAS (Figure 2). RAS is a membrane-bound GTPase that cycles between an inactive guanosine diphosphate (GDP)-bound form and an active guanosine triphosphate (GTP)-bound form. As a binary cell switch, RAS protein is activated by extracellular stimulation [63–66]. KRAS is located on the inner surface of the cell membrane and it transmits signals from activated transmembrane receptor EGFR to effectors in the MAPK and type I phosphatidylinositol 3-kinase (PI3K)/v-aktmurine-thymomaviral oncogene (AKT)-signaling pathway in the cytoplasm [10, 67]. On one side, active GTP-bound RAS binds to the three closely related RAF proteins, CRAF1, BRAF, and ARAF, causing RAF to be relocated to the plasma membrane, triggering engagement of the pathway [68, 69]. Activated RAF changes the phosphorylation status and activates MEK1 and MEK2. MEK are capable of phosphorylating and activating the MAPK

Table 3. *BRAF* and *KRAS* mutations as prognostic factors in clinical studies of first-line treatment for mCRC

Name of study	Phase of clinical trial	Therapeutic strategy	Key outcomes in patients	Prognostic finding
MRC COIN	III	OFC	Median OS, 8.8 vs 20.1 months for patients with wild-type *BRAF* ($P < 0.001$); median OS, 14 vs 20.1 months for patients with wild-type *KRAS* ($P < 0.001$)	*KRAS* and *BRAF* mutations have a strong prognostic effect
CRYSTAL	III	FOLFIRI, cetuximab	Median OS, 8.8 vs 20.1 months for patients with wild-type *BRAF* ($P < 0.001$); median OS, 14 vs 20.1 months for patients with wild-type *KRAS* ($P < 0.001$)	*KRAS* and *BRAF* mutations are important indicators of poor prognosis
PRIME	III	FOLFOX4, panitumumab	In the mutant *KRAS* stratum, PFS was significantly reduced in the panitumumab–FOLFOX4 arm vs the FOLFOX4 arm; median OS, 15.5 vs 19.3 months, respectively ($P = 0.068$)	*KRAS* testing is important for patients with mCRC
CAIRO2	III	CBC	Median PFS, 8.1 and 12.5 months in patients with *KRAS*-mutant vs 10.5 and 10.6 months in patients with *KRAS* wild-type tumors in two arms Median OS, 17.2 and 24.9 months in patients with *KRAS*-mutant tumors vs 21.8 and 22.4 months in patients with *KRAS* wild-type tumors with CB and CBC, respectively	Mutation status of the *KRAS* gene was a predictor of outcome in the cetuximab group
CAIRO2	III	CBC	Median PFS, 5.9 and 6.6 months in patients with *BRAF*-mutant tumors vs 12.2 and 10.4 months in patients with *BRAF* wild-type tumors in two arms Median OS, 15.0 and 15.2 months in *BRAF*-mutant vs 24.6 and 21.5 months in *BRAF* wild-type tumors in two arms	*BRAF* mutation was a negative prognostic marker in patients with mCRC
NORDIC-VII	III	Nordic FLOX, cetuximab	ORRs, 20% in patients with *BRAF*-mutant tumors vs 50% in those with *BRAF* wild-type tumors ($P < 0.01$)	Presence of *BRAF* mutations was a strong negative prognostic factor
TAILOR	III	FOLFOX-4, cetuximab	Median PFS, 2.0 in patients with *BRAF*-mutant tumors vs 9.3 months in patients with *BRAF* wild-type tumors	Mutation status of the *BRAF* gene was a predictor of outcome in the cetuximab group
Pooled analysis of CAIRO, CAIRO2, COIN, and FOCUS phase 3 studies	III	CBC	Median PFS, 6.2 in *BRAF*-mutant vs 7.7 months in *BRAF* wild-type tumors Median OS, 11.4 in *BRAF*-mutant vs 17.2 months in *BRAF* wild-type tumors	*BRAF*-mutant status was a biomarker conferring poor prognosis in mCRC

BRAF, V-raf murine sarcoma viral oncogene homolog B1; KRAS, V-Ki-ras2 Kirsten ratsarcoma viral oncogene homolog; OFC, oxaliplatin, fluoropyrimidine, cetuximab; FOLFIRI, irinotecan, infusional fluorouracil, leucovorin; FOLFOX4, infusional fluorouracil, leucovorin, and oxaliplatin; CBC, capecitabine, oxaliplatin, bevacizumab, cetuximab.

extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) and regulate the activity of several transcription factors that induce the expression of multiple genes involved in cell survival and proliferation [70–72]. The other effector pathway is the PI3K/AKT pathway. RAS can interact directly with the catalytic subunit of PI3Ks. PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate to produce phosphatidylinositol 3,4,5-trisphosphate that can bind to a large number of proteins as a second messenger through the pleckstrin homology domain and other domains such as AKT [73, 74].

Constitutive activation of the MAPK-signaling pathway is a major cause of resistance to anti-EGFR therapies in *KRAS*- and *BRAF*-mutant mCRC. Mutations of the *RAS* genes themselves, most frequently *KRAS* mutations, contribute to aberrant signaling through the RAS pathways. This weakens the GTPase-activating proteins (GAPs) and increases the intrinsic catalytic rate of GTPase and prevents the hydrolysis of GTP to GDP, causing an accumulation of active GTP-bound RAS. Accumulation of GTP-bound RAS leads to constitutive KRAS activity, triggering downstream signal transduction and promoting survival and proliferation of the cancer cell. Almost all RAS activation in tumors is caused by mutations in codons 12, 13, and 61 [7, 75–79]. As a downstream signaling protein of RAS, RAF kinases normally play an important role in the EGFR-mediated MAPK pathway [13, 24]. Some (4%–15%) of mCRC tumors bear *BRAF* mutations and most of them are V600E amino-acid substitutions [11, 25]. As a central amino acid in the kinase domain, V600 is required to maintain RAF in its inactive conformation without RAS activation. Mutations in *BRAF* V600 can constitutively activate the MAPK pathway, regardless of whether RAS is activated or not, resulting in anti-EGFR resistance [54]. This receptor-independent pathway activation makes cancerous cells unresponsive to anti-EGFR molecules.

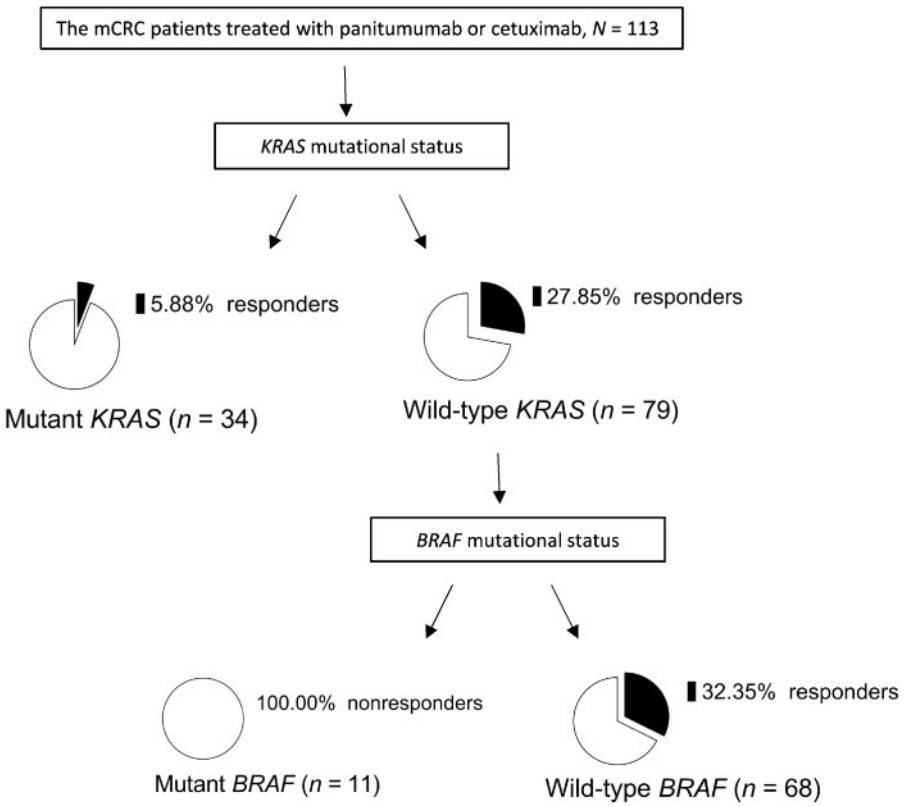

Figure 1. *KRAS* and *BRAF* mutations are prognostic and predictive biomarkers in anti-EGFR combined regimens for mCRC treatment. *KRAS* and *BRAF* mutations are associated with poor response to treatment with anti-EGFR. In the sample set, none of the patients harboring the *KRAS* mutation carried the associated *BRAF* mutation, indicating their exclusive relationship in mCRC. The data in the figure are from a clinical trial mentioned in the corresponding part of the text [37].

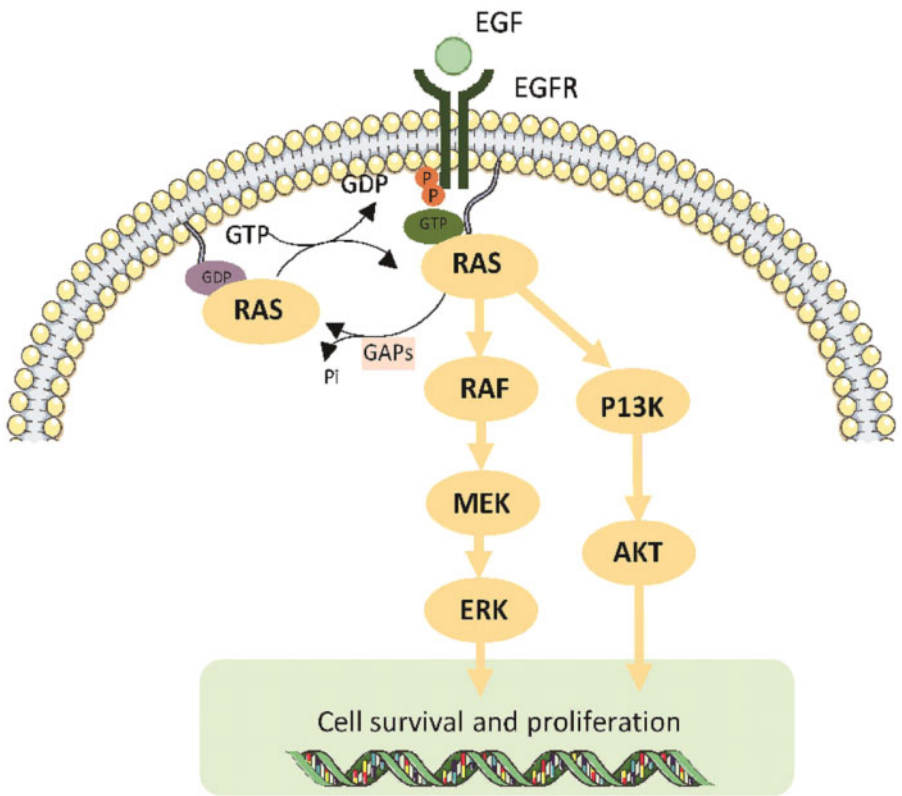

Figure 2. The downstream signaling pathway of the EGFR mainly. EGF selectively binds to EGFR and triggers the receptor to form a dimer that activates RAS. RAS transmits signals from activated transmembrane receptor EGFR to effectors in the MAPK- andPI3K/AKT-signaling pathway in the cytoplasm. Active GTP-bound RAS triggers RAF to engage in signal transmission. Activated RAF changes the phosphorylation status and activates MEK1 and MEK2. MEK are capable of phosphorylating and activating the MAPK extracellular signal ERK1 and ERK2, and regulate cell survival and proliferation. The other effector pathway is the PI3K/AKT pathway. RAS can interact directly with the catalytic subunit of PI3Ks and activate it; activated PI3Ks trigger AKT participate in signal transmission.

Furthermore, alterations in additional nodes of the EGFR pathway also appear to confer primary anti-EGFR resistance. Studies have demonstrated that mutations in *PIK3CA* exon 20 and phosphatase and tensin homolog (*PTEN*) alterations frequently coexist with *RAS* mutations. These alterations are associated with resistance to anti-EGFR mAbs [62, 80]. Amplification of *KRAS* and the MET receptor have been shown to bypass EGFR signaling and activate the EGFR pathway [81–83]. MET signaling seems to have a synergistic effect on the EGFR pathway in promoting the growth of tumor cells [84]. Moreover, the extensive crosstalk among the ERBB (also known as HER) family of receptors can up-regulate parallel pathways as a compensatory adaptive mechanism when EGFR is inhibited. EGFR can form heterodimers with the receptor tyrosine-protein kinase erbB-3 (HER3) and active the downstream PI3K- and MAPK-signaling pathways [85–87].

Subsequent studies found that some patients with wt-*KRAS* and wt-*BRAF* mCRC initially respond to anti-EGFR therapies; a few patients experience disease progression eventually. This is indicative of acquired resistance to anti-EGFR therapy. The most common mechanisms that drive secondary resistance to anti-EGFR agents are genetic alterations that contribute to primary resistance: *KRAS*, *NRAS*, *BRAF*, and *PIK3CA* mutations. These mutations were found in biopsies of patients who experienced relapse, although these mutations were not observed in patients at the beginning of treatment [88–91]. Biopsies confirmed that the number of mutant alleles increased under drug exposure and became undetectable after drug withdrawal [92]. Another mechanism of secondary-resistance acquisition is the prevention of drug binding to the receptor by acquired *EGFR* extracellular domain mutations (exon 12). S492R, a particular acquired *EGFR* mutation, is associated with secondary resistance to cetuximab in mCRC. The S492R mutation within the EGFR ectodomain perturbs the conformational state of the kinase drug-binding sites and the resulting bulky side-chain structure at this position could interfere with cetuximab binding [93–95]. *ERBB2* gene upregulation has also been proposed as a factor of secondary resistance to anti-EGFR therapies in wt-*RAS* and wt-*BRAF* mCRC. Activated HER2 signaling in cancer cells can promote *HER2* transcription or upregulate the HER2/HER3 ligand heregulin, thus conferring resistance to anti-EGFR therapies [96, 97].

Emerging therapies targeting *BRAF*-mutant mCRC

Combining *BRAF* and EGFR inhibitors

BRAF mutations commonly occur in melanoma and the *BRAF* inhibitor vemurafenib elicits a good response in 60%–80% of patients with *BRAF*-mutant melanoma in clinical trials [98, 99]. Trials testing the efficacy of this *BRAF* inhibitor in the treatment of *BRAF*-mutation-bearing patients with mCRC indicated that vemurafenib gives a poor prognosis for this type of patient [100, 101]. Further investigation revealed that the resistance is induced by reactivation of the *CRAF*-mediated MAPK signal. As shown in Figure 3, when *BRAF*–MEK–ERK signaling is interrupted by the *BRAF* inhibitor, the signaling output from mutant *BRAF* is blocked, causing transient suppression of ERK activation. ERK-dependent negative feedback activates EGFR, causing RAF protein dimer formation and reactivation of the *CRAF*-mediated MAPK signaling, which can bypass the *BRAF* node [27, 67, 98]. On the one hand, this mechanism explains why the single *BRAF* inhibitor has an antitumor effect in melanoma but not in mCRC. Melanoma cells express low levels of EGFR, so they are not influenced by this feedback activation and therefore demonstrate a good response to the single-agent *BRAF* inhibitor [98]. In contrast, these findings suggest that there is a strong

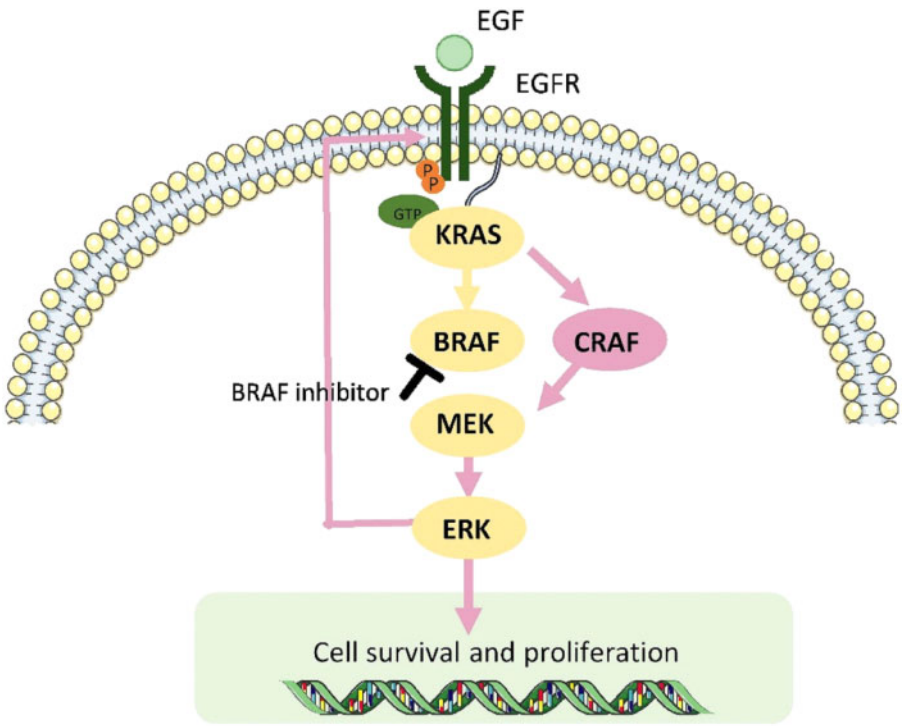

Figure 3. The resistance mechanism of BRAF inhibitors. BRAF inhibition interrupts the RAF–MEK–ERK signaling, releases ERK-dependent negative feedback on the EGFR, and subsequently activates EGFR, formats the RAF protein dimer, then reactivates the CRAF-mediated MAPK signal.

Table 4. Advanced combining therapies on BRAF-mutated mCRC

Therapeutic strategy	Agents investigated	PFS in the experiment group (months)	PFS in the control group (months)
Combining *BRAF* and EGFR inhibitors	Vemurafenib + panitumumab	3.2	~2 months (standard therapies); 2.1 (singlet *BRAF* inhibitor)
	Dabrafenib + panitumumab	3.5	
	Encorafenib + cetuximab	3.7	
Combining *BRAF* and MEK inhibitors	Dabrafenib + trametinib	3.5	
Combining EGFR and MEK inhibitors	Panitumumab + trametinib	2.6	
Combining *BRAF*, EGFR, and MEK inhibitors	Dabrafenib + trametinib + panitumumab	4.2	2.6/3.5
	Encorafenib + cetuximab + binimetinib	4.3	1.5

dependency on MAPK signaling in mCRC and adequate inhibition of this pathway may improve response to treatment. A combination of *BRAF* inhibitors and anti-EGFR mAbs has been shown to produce sustained suppression of MAPK signaling and overcome EGFR-driven resistance *in vitro* and in xenograft models [102–105]. This indicates that doublet-targeted therapy may overcome the feedback effect. The various combination therapies are shown in Table 4. A combination of panitumumab (EGFR inhibitor) and vemurafenib (*BRAF* inhibitor) treatment in *BRAF*-mutant mCRC demonstrated antitumor efficacy: PFS was 3.2 months [95% confidence interval (CI), 1.6–5.3] and OS was 7.6 months (95% CI, 2.1–not reached). Compared with patients on single-agent vemurafenib or panitumumab, patients undergoing double therapy displayed decreased incidence and severity of acneiform rash (40% of grade 1 and 13% of grade 2), maculopapular rash (13%), palmar-plantar erythrodysesthesia syndrome (7%), papilloma (7%), and cutaneous squamous cell carcinoma/keratoacanthoma (0%), likely due to the opposing effects of vemurafenib (activation) and panitumumab (inhibition) on ERK signaling in epidermal keratinocytes [106]. A clinical trial evaluating dabrafenib plus panitumumab reported similar results: the 20 patients enrolled had a median PFS of 3.5 months (95% CI, 2.8–5.8 months) and OS of 13.2 months (95% CI, 6.7–22.0 months) [107]. This therapy was well tolerated and the majority of adverse events were grade 1 or 2. The most common adverse events of any grade were acneiform dermatitis (60%), nausea (50%), fatigue (50%), and diarrhea (45%) [107]. Other combinations of *BRAF* inhibitors and EGFR inhibitors are also in clinical trial; the PFS for encorafenib plus cetuximab was 3.7 months. The most common treatment-related adverse events were as follows: all grades [fatigue (46%), infusion-related reaction (35%)], and grade 3/4 [fatigue (8%), hypophosphatemia (15%)] [108]. Compared with the PFS of patients treated with singlet *BRAF* inhibitor (2.1 months, 95% CI, 0.4–11.6), the PFS of patients treated with combined *BRAF* and EGFR inhibitors significantly improved [109].

Combining *BRAF* inhibitors and MEK inhibitors

Studies revealed that *BRAF* inhibitors can interrupt the signaling output from mutant *BRAF* and reactivate the *CRAF*-mediated MAPK signal, bypassing the *BRAF* node to transmit signaling to MEK. Therefore, MEK inhibitors may suppress the *CRAF*-mediated MAPK pathway. Mature strategies combining the *BRAF* inhibitor dabrafenib and MEK inhibitor trametinib have been successful in *BRAF*-mutant melanoma and *BRAF*-mutant non-small-cell lung cancer (NSCLC), and significantly improved outcomes compared with those patients treated with dabrafenib alone [2, 110–114]. The doublet combination of dabrafenib and trametinib for *BRAF*-mutant mCRC has also been trialed in clinic. Combined dabrafenib and trametinib contribute to an improved response rate (median PFS, 3.5 months; 95% CI, 3.4–4.0 months). In terms of safety, 17 adverse events (40%) led to a dose reduction, 25 adverse events (58%) led to a dose interruption, and four patients (9%) discontinued treatment due to an adverse event [115]. Another clinical trial of the same drug combination reported similar results. At data cut-off, 3-year PFS rate was 22% for the doublet-treatment group and 12% for the singlet-treatment group (HR, 0.71; 95% CI, 0.57–0.88), and the 3-year OS rate was 44% and 32%, respectively (HR, 0.75; 95% CI, 0.58–0.96). The incidence of adverse events was higher (>10% difference, any grade) in the doublet-treatment group than in the singlet-treatment group: pyrexia (59% vs 33%), chills (32% vs 17%), diarrhea (31% vs 17%), vomiting (26% vs 15%), and peripheral edema (22% vs 9%) [116]. Combining *BRAF* and MEK inhibitors has similar efficacy to combining *BRAF* and EGFR inhibitors, and results in improved PFS compared with singlet *BRAF* inhibitors.

Combining EGFR inhibitors and MEK inhibitors

Combined MEK and EGFR inhibition with trametinib and/or panitumumab for *BRAF*-mutant mCRC has also been trialed in clinic. No patient in the trametinib-plus-panitumumab-treatment group achieved complete or partial response and 55% were stable (T + P, $n = 31$), whereas, in the dabrafenib-plus-panitumumab-treatment group (D + P, $n = 20$), 10% had a confirmed complete or partial response and 80% were stable. The triplet-treatment group (D + P plus trametinib, $n = 91$) resulted in a confirmed complete response or partial response in 21% of patients, stable disease in 65%, and an overall disease-control rate of 86%. The median PFS in the trametinib-plus-panitumumab group was 2.6 months (95% CI, 1.4–2.8 months) compared with 3.5 months (95% CI, 2.8–5.8 months) in the D + P group and 4.2 months (95% CI, 4.0–5.6 months) in the D + P plus trametinib group. These results suggest that combination therapy of *BRAF* and EGFR inhibitors may not be a priority in *BRAF*-mutant mCRC [107, 117].

The triplet chemotherapy of *BRAF*, EGFR, and MEK inhibitors

A clinical trial compared the combination of dabrafenib and panitumumab with or without trametinib in 120 patients with *BRAF*-mutant mCRC. PFS was 4.2 months (95% CI, 4.0–5.6 months) in the triplet-treatment group and 3.5 months (95% CI, 2.8–5.8 months) in the treatment group without trametinib [107]. A large phase III trial involving only patients with *BRAF*–V600E-mutated mCRC was recently reported. A total of 665 patients were divided into three equal groups: triple-therapy

group (encorafenib, binimetinib, and cetuximab), double-therapy group (encorafenib and cetuximab), and a control group (the investigators' choice of either cetuximab and irinotecan or cetuximab and FOLFIRI). The therapeutic effect was significant; the median OS was 9.0 months (95% CI, 8.0–11.4) in the triple-therapy group, 8.4 months (95% CI, 7.5–11.0) in the double-therapy group, and 5.4 months (95% CI, 4.8–6.6) in the control group. Additionally, the risk of death was significantly lower (by 48%) in the triple-therapy group than in the control group (HR, 0.52; 95% CI, 0.39–0.70; $P < 0.001$). The PFS was significantly longer in both the triple-therapy group and the double-therapy group than in the control group: the median PFS was 4.3 months (95% CI, 4.1–5.2) in the triple-therapy group, 4.2 months (95% CI, 3.7–5.4) in the double-therapy group, and 1.5 months (95% CI, 1.5–1.7) in the control group [118]. Importantly, no increase in skin toxicity or fatigue was observed with the triple combination and no new safety signals were noted.

Standard therapies have limited benefit for *BRAF*-mutation-bearing patients with mCRC with a PFS of approximately 2 months and an OS of 4–6 months [118]. Double and triple therapies can both prolong PFS; triple therapy combining BRAF, EGFR, and MEK inhibitors remarkably delivers a much longer PFS than the double therapy (Table 4).

Combination of MEK inhibitors and ERK inhibitors

Studies have evaluated molecular alterations in the MAPK pathway after treatment with a BRAF/EGFR inhibitor or *BRAF*/MEK inhibitor in *BRAF*-mutant mCRC [29]. The mechanism of resistance involved *KRAS* mutation, *KRAS* upregulation, *BRAF* upregulation, and *MEK1* mutation. The use of MEK inhibitors triggers *KRAS* mutation, *KRAS* upregulation, and *BRAF* upregulation, thus increasing the pathway flux and promoting MEK hyperactivation that can overcome the efficacy of MEK inhibitors [38, 81]. Despite upstream MAPK-pathway alterations, ERK inhibitors still have the ability to suppress the reactive MAPK pathway [119, 120]. Studies on the effects of the ERK-inhibitor combination strategy on a *BRAF*-mutant mCRC cell line suggest that the combination of ERK inhibitors and MEK inhibitors is more effective than single treatment [119, 120]. Although ERK inhibitors are at the early stages of clinical trial, they are expected to play an important role in the advanced therapeutic regimen for *BRAF*-mutant mCRC [121].

Emerging therapies targeting *KRAS*-mutant mCRC

KRAS mutations occur in 35%–45% of mCRC, causing therapy resistance and poor prognosis. *KRAS* is regarded as an 'undruggable' oncoprotein that cannot be targeted pharmacologically, so we discuss recent progress in new directions such as the parallel inhibition of the PI3K/AKT and MAPK pathways, directly targeting mutant *KRAS*, targeting *KRAS*-membrane association, exploring the *KRAS*-regulated metabolic pathway, as well as immunotherapy and *KRAS* synthetic lethal interactions [72, 122–124].

Combination of PI3K inhibitor and MEK inhibitor in preclinical trial

Although *KRAS* cannot be directly targeted, we can target downstream RAS and parallel signaling pathways. PI3K functions downstream to RTKs, which are regulated by RAS kinases. MEK and PI3K are parallel signaling pathways. Inhibition of the MEK pathway activates the PI3K pathway, and PI3K activation mediates resistance to MEK inhibition [125]. A study tested whether combining MEK and PI3L inhibitors can produce a stronger response in a *KRAS*-mutant cell line. The study assessed a combination of an MEK1/2 inhibitor AZD6244 and a PI3K inhibitor BEZ235 *in vivo* and measured the tumor volumes of tumor-bearing mice. Treatment with cetuximab for 3 weeks did not inhibit tumor growth; treatment with AZD6244 or BEZ235 alone caused a ~50% reduction in tumor growth. Notably, the combined treatment of AZD6244 and BEZ235 completely inhibited tumor growth at the end of the 3-week treatment period. Importantly, there were no signs of weight loss or other acute or delayed toxicity symptoms in either the single-agent- or combination-treatment group [126]. This preclinical trial provides convincing evidence that combined PI3K inhibitors and MEK inhibitors enhance antitumor efficacy against *KRAS*-mutant cells, which strongly supports the development of clinical trials for this patient population.

Directly targeting *KRAS*-mutant mCRC

RAS is a membrane-bound GTPase that cycles between an inactive GDP-bound form and an active GTP-bound form. RAS protein is activated by extracellular stimulation and is converted to active GTP-bound RAS. *KRAS* G12C is one of the most common *KRAS* mutants in cancer, present in 10%–20% of all *KRAS* G12 mutations. Biochemical analysis showed that KRAS G12 preferentially binds to the inactive GDP-bound form of RAS, impairing SOS-catalysed nucleotide exchange and decreasing the affinity of RAS for GTP, resulting in decreased survival and increased apoptosis of tumor cells containing the G12C oncogene [127–129].

AMG 510, the first KRAS G12C inhibitor in clinical development, has undergone preclinical trial [130]. AMG 510 treatment reduced tumor size and, in some cases, even eradicated the tumor in a *KRAS*-mutant murine model. Moreover, AMG 510 has a synergistic effect with chemotherapy, immunotherapy, and other targeted inhibitors such as the MEK inhibitor. In the NCI-H358 tumor-cell line carrying the *KRAS* G12C mutation, AMG 510 had synergistic effects with a MEK inhibitor. *In vivo*, the combination of low-dose AMG 510 and MEK inhibitor significantly improved the antitumor effect compared with single-drug therapy in tumor-bearing mice. Tumor growth was inhibited in singlet-treatment groups compared with the control group, and the tumor volume tended to decrease in the combination-treatment group. These data suggest that the combination of AMG 510 with inhibitors targeting the MAPK-signaling pathway may eliminate bypass or residual signaling, which may in turn enhance efficacy or prevent drug resistance. When AMG 510 is combined with carboplatin, a standard chemotherapy for lung cancer *in vivo*, the combined treatment significantly reduced tumor volume compared with single-drug therapy. Additionally, tumor-bearing mice were treated with suboptimal doses of AMG 510 and/or PD-1 inhibitors, which can upregulate T-cell activity and enhance the T-cell recognition of tumor cells. The results showed that AMG 510 single-drug treatment caused the complete disappearance of the tumor in only 1 of 10 mice and PD-1-inhibitor monotherapy had a similar effect, whereas the combination therapy resulted in the complete disappearance of tumors in 9 of 10 mice. This indicates that AMG 510 has potential as a combination therapeutic agent in *KRAS*-mutant mCRC.

Targeting G-quadruplex (G4) structures of the KRAS gene

G4 structures are formed in guanine-rich nucleic-acid sequences and play a key role in tumor development through the regulation of a wide range of oncogenes, tumor-suppressor genes, and somatic copy-number alterations [131, 132]. There are three G4 motifs in the human *KRAS* promoter: G4-proximal, G4-middle, and G4-distal. In particular, the G4-middle structure may play an important part in the development of targeted therapeutics [133, 134]. EMICORON, a novel synthetic G4 ligand, has shown promising antitumor efficacy against CRC tumors. In HCT-116 CRC cells, *KRAS* mRNA and protein expression were downregulated after EMICORON treatment, suggesting that EMICORON can downregulate both *KRAS* mRNA and protein expression by targeting the G4 structure. *In vivo*, EMICORON was tested on a panel of patient-derived xenografts (PDXs) bearing *KRAS* mutations. In all three PDXs, the tumor volume of mice treated with EMICORON was decreased compared with that of untreated mice. In addition, no adverse effects were observed in the treated mice. EMICORON can also improve the efficacy of chemotherapy: CRC PDXs treated with EMICORON plus FOLFIRI showed better response rates and survival than the FOLFIRI-alone-treatment group [135, 136]. These results suggest that EMICORON has therapeutic potential for *KRAS*-mutant mCRC.

Potential therapeutic strategies in *KRAS*-mutant mCRC

Targeting *KRAS*-membrane association

KRAS located on the inner leaflet of the plasma membrane transmits signals from activated EGFR transmembrane receptors to downstream cytoplasmic effectors. *KRAS* realizes its oncogenic activity through this membrane association. Therefore, inhibiting *KRAS*-membrane association seems a logical therapy for *KRAS*-mutant cancers [137]. Phosphodiesterase-6δ (PDEδ) is a prenyl-binding protein that enriches RAS at the plasma membrane, thus augmenting RAS signaling [138]. A small-molecule inhibitor, deltarasin, prevents PDEδ from binding to *KRAS* and impairs *KRAS* localization to the endomembrane. Deltazinone 1, an improved PDEδ inhibitor, showed high selectivity and less nonspecific cytotoxicity than deltarasin and, at the same time, exhibited high correlation with the phenotypic effect of PDEδ knockdown in a set of human pancreatic-cancer-cell lines [139, 140]. However, further chemical optimization is required, as its chemical properties are unstable *in vivo*.

Targeting the KRAS-regulated metabolic pathway

The infinite proliferation of tumor cells requires metabolic reprogramming to produce biomass. *KRAS*-mutant tumors also develop the metabolic pathways to obtain substrates from both extracellular and intracellular sources to meet their metabolic need [141, 142]. *KRAS*-mutant CRC tumors show high expression of glycolytic and glutamic metabolic proteins [143]. CB-839—an inhibitor targeting the glutaminase (GLS) that contributes to the conversion of glutamine to glutamate—is currently being evaluated in phase I and II clinical trials for the treatment of various cancers [144]. In addition, *KRAS*-mutant cells show glyceraldehyde 3-phosphate dehydrogenase (GAPDH) metabolic vulnerability compared with wt-*KRAS* cells and vitamin C selectively kills *KRAS*-mutant CRC cells by inhibiting GAPDH [145].

Immunotherapy and synthetic lethal interactions in *KRAS*-mutant cancer

Immunotherapy is a key focus in cancer treatment currently, especially immune-checkpoint-blocking antibodies. Immune-checkpoint-blocking antibodies are profoundly changing the therapeutic strategies of various cancers. Specifically, the anti-PD-1/PD-L1 antibody has shown significant antitumor activity by restoring the T-cell antitumor response. PD-L1 is present in some normal cells as well as cancer cells. When PD-1 is bound to PD-L1, T-cells do not attack the cell. Some cancer cells contain large amounts of PD-L1, allowing them to escape immune attack. mAbs targeting PD-1 or PD-L1 can block this binding and prevent cancer cells from escaping T-cell attack, thereby restoring the normal immune response to cancer cells. Anti-PD-1/PD-L1 antibody treatment is now approved in various tumor types [146]. *KRAS*-mutant NSCLC was effectively treated with the PD-1 antibody pembrolizumab combined with the MEK inhibitor trametinib [147]. Clinical trials demonstrated that the PD-1 antibody treatment is more effective in patients with *KRAS*-mutant NSCLC than in patients with wt-*KRAS* NSCLC [147, 148]. This is likely because *KRAS* mutations impede DNA repair, especially mismatch repair (MMR), which supports the notion that MMR deficiency is a favorable factor for PD-1 blockade [149]. This result suggests that immunotherapy is a promising potential treatment strategy for *KRAS*-mutant mCRC.

Synthetic lethal interactions in *KRAS*-mutant cancer have also been widely studied. Synthetic lethality defines the interaction between two co-essential genes such that inhibiting both genes rather than either single gene can result in cell death [150]. There are several stage I and II clinical trials assessing the synthetic lethality of MEK inhibitors combined with BCL2, AKT, or SHP2 in *KRAS*-mutant cancer. These treatments produce antitumor efficacy by interrupting the pathways required for the survival of *KRAS*-mutant cells, including co-operating signaling, transcriptional regulation, and the maintenance of genomic stability [151–153]. We found that most of the synthetic lethality studies in *KRAS*-mutant cancer are focused on specific cancer cell lines or tumor models; however, as *KRAS*-mutant cancers in clinical trials are highly heterogeneous, further studies should aim at systematically examining a spectrum of *KRAS*-mutant cancers.

Combination of curcumin and regorafenib

Curcumin is a polyphenol, which is the main yellow pigment in turmeric. Pharmacologically, it has an anticancer effect in human clinical trials by promoting apoptosis and inducing autophagy [154]. Additionally, in an ongoing phase III clinical trial, curcumin has been demonstrated to be a safe and effective treatment for patients with CRC [155]. Regorafenib is a multityrosine kinase inhibitor that mainly inhibits antigenic and oncogenic kinases, such as VEGFR, PDGFR, FGFR, and BRAF. Two randomized phase III clinical studies (CORRECT and CONCUR) evaluated the efficacy of regorafenib in mCRC treatment and the results indicate that regorafenib is a promising treatment option for patients with mCRC who had poor prognosis [156–158].

Another study found that curcumin had a similar antitumor effect to MEK inhibitor U0126 and that this effect was enhanced when curcumin was used synergistically with regorafenib in the *KRAS*-mutant CRC cell line HCT 116. Inhibiting MEK conferred the synthetic lethal interaction with RAF1 inhibition in a new concept for combination-drug treatments. It may be possible to

overcome the limited therapeutic effect of current treatments for *KRAS*-mutant mCRC by developing doublet-combination therapies targeting the RAF–MEK pathway. Given that curcumin has the potential to inhibit MEK and regorafenib is a RAF-targeting agent, we propose that the combination of curcumin and regorafenib is a promising therapeutic strategy for *KRAS*-mutant mCRC [159, 160].

Conclusions

Personalized therapy enables patients to receive maximum treatment efficacy with minimum side effects, ensuring the best possible quality of life and maximum survival of patients. The current approved first-line combination of chemotherapy and anti-EGFR agent treatment that has been highly effective in patients with mCRC clinically has little benefit and poor prognosis when applied to *BRAF*- and *KRAS*-mutation-bearing patients with mCRC. Analysis of the *BRAF*- or *KRAS*-mutant occurrence in no-response-to-anti-EGFR-agents patients and possible resistance mechanisms suggest that *BRAF*- and *KRAS*-mutant statuses are critical prognostic and predictive biomarkers in mCRC treatment. Utilizing this biomarker status to select patients and formulate personalized therapeutic strategies according to oncogene status promises to improve patient outcomes. The addition of an inhibitor of the specific *BRAF* mutation provides additional treatment options that include EGFR inhibitors and increases the likelihood of treatment-delayed disease progression. Combining inhibitors that target specific node kinases such as EGFR inhibitors, BRAF inhibitors, MEK inhibitors, and ERK inhibitors provides promising clinical treatment to delay disease progression and prolong survival times. While no mature inhibitor-targeted therapies for *KRAS*-mutant mCRC have been currently adopted into clinical treatment, data from preclinical and clinical trials indicate potential combination-treatment options for *KRAS*-mutant mCRC patients such as curcumin plus regorafenib or PI3K inhibitor plus MEK inhibitor.

Authors' contributions

Z.N.L. and L.Z. wrote the manuscript, L.F.Y. contributed to the manuscript, and M.J.W. reviewed the final versions. All authors read and confirmed the final version of the manuscript.

Funding

This work was supported by National Natural Science Foundation of China [No. U1608281]; National Natural Science Foundation of China [81903658]; Liaoning Province Scientific Research Foundation [JC2019032]; Liaoning Revitalization Talents Program [No. XLYC1807201]; and Shenyang S&T Projects [19–109-4–09].

Conflicts of interest

None declared.

References

1. Bylsma LC, Gillezeau C, Garawin TA *et al*. Prevalence of RAS and BRAF mutations in metastatic colorectal cancer patients by tumor sidedness: a systematic review and meta-analysis. *Cancer Med* 2020;**9**:1044–57.
2. Robert C, Karaszewska B, Schachter J *et al*. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med* 2015;**372**:30–9.
3. Cremolini C, Schirripa M, Antoniotti C *et al*. First-line chemotherapy for mCRC—a review and evidence-based algorithm. *Nat Rev Clin Oncol* 2015;**12**:607–19.
4. Martini G, Troiani T, Cardone C *et al*. Present and future of metastatic colorectal cancer treatment: a review of new candidate targets. *World J Gastroenterol* 2017;**23**:4675–88.
5. Punt CJ, Tol J. More is less—combining targeted therapies in metastatic colorectal cancer. *Nat Rev Clin Oncol* 2009;**6**:731–3.
6. Downward J. The ras superfamily of small GTP-binding proteins. *Trends Biochem Sci* 1990;**15**:469–72.
7. Scheffzek K, Ahmadian MR, Kabsch W *et al*. The Ras-RasGAP complex: structural basis for GTPase activation and its loss in oncogenic Ras mutants. *Science* 1997;**277**:333–8.
8. Saridaki Z, Androulakis N, Vardakis N *et al*. A triplet combination with irinotecan (CPT-11), oxaliplatin (LOHP), continuous infusion 5-fluorouracil and leucovorin (FOLFOXIRI) plus cetuximab as first-line treatment in KRAS wt, metastatic colorectal cancer: a pilot phase II trial. *Br J Cancer* 2012;**107**:1932–7.
9. Allegra CJ, Jessup JM, Somerfield MR *et al*. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. *J Clin Oncol* 2009;**27**:2091–6.
10. Tomasini P, Walia P, Labbe C *et al*. Targeting the KRAS pathway in non-small cell lung cancer. *Oncologist* 2016;**21**:1450–60.
11. Mao C, Wu XY, Yang ZY *et al*. Concordant analysis of KRAS, BRAF, PIK3CA mutations, and PTEN expression between primary colorectal cancer and matched metastases. *Sci Rep* 2015;**5**:8065.
12. Poulikakos PI, Zhang C, Bollag G *et al*. RAF inhibitors transactivate RAF dimers and ERK signalling in cells with wild-type BRAF. *Nature* 2010;**464**:427–U126.
13. Cicenas J, Tamosaitis L, Kvederaviciute K *et al*. KRAS, NRAS and BRAF mutations in colorectal cancer and melanoma. *Med Oncol* 2017;**34**:26.
14. Chong H, Vikis HG, Guan KL. Mechanisms of regulating the Raf kinase family. *Cell Signal* 2003;**15**:463–9.
15. Hancock JF. Ras proteins: different signals from different locations. *Nat Rev Mol Cell Biol* 2003;**4**:373–84.
16. Prieto-Potin I, Montagut C, Bellosillo B *et al*. Multicenter evaluation of the Idylla NRAS-BRAF mutation test in metastatic colorectal cancer. *J Mol Diagn* 2018;**20**:664–76.
17. Fernandez-Medarde A, Santos E. Ras in cancer and developmental diseases. *Genes Cancer* 2011;**2**:344–58.
18. Douillard JY, Oliner KS, Siena S *et al*. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med* 2013;**369**:1023–34.
19. Heinemann V, von Weikersthal LF, Decker T *et al*. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2014;**15**:1065–75.
20. Schwartzberg LS, Rivera F, Karthaus M *et al*. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. *J Clin Oncol* 2014;**32**:2240–7.
21. De Roock W, Claes B, Bernasconi D *et al*. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of

cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010;**11**:753–62.
22. Peeters M, Oliner KS, Parker A *et al*. Massively parallel tumor multigene sequencing to evaluate response to panitumumab in a randomized phase III study of metastatic colorectal cancer. *Clin Cancer Res* 2013;**19**:1902–12.
23. Guo F, Gong H, Zhao H *et al*. Mutation status and prognostic values of KRAS, NRAS, BRAF and PIK3CA in 353 Chinese colorectal cancer patients. *Sci Rep* 2018;**8**:6076.
24. Davies H, Bignell GR, Cox C *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002;**417**:949–54.
25. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. *J Clin Oncol* 2010;**28**:1254–61.
26. Cancer Genome Atlas N. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**:330–7.
27. Corcoran RB. New therapeutic strategies for BRAF mutant colorectal cancers. *J Gastrointest Oncol* 2015;**6**:650–9.
28. Wan PTC, Garnett MJ, Roe SM *et al*. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004;**116**:855–67.
29. Ahronian LG, Sennott EM, Van Allen EM *et al*. Clinical acquired resistance to RAF inhibitor combinations in BRAF-mutant colorectal cancer through MAPK pathway alterations. *Cancer Discov* 2015;**5**:358–67.
30. Richman SD, Seymour MT, Chambers P *et al*. KRAS and BRAF mutations in advanced colorectal cancer are associated with poor prognosis but do not preclude benefit from oxaliplatin or irinotecan: results from the MRC FOCUS trial. *J Clin Oncol* 2009;**27**:5931–7.
31. Maughan TS, Adams RA, Smith CG *et al*. Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011;**377**: 2103–14.
32. Ducreux M, Chamseddine A, Laurent-Puig P *et al*. Molecular targeted therapy of BRAF-mutant colorectal cancer. *Ther Adv Med Oncol* 2019;**11**:175883591985649. 1758835919856494.
33. Tie J, Gibbs P, Lipton L *et al*. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer* 2011;**128**: 2075–84.
34. Tol J, Koopman M, Cats A *et al*. Chemotherapy, bevacizumab, and cetuximab in metastatic colorectal cancer. *N Engl J Med* 2009;**360**:563–72.
35. Hecht JR, Mitchell E, Chidiac T *et al*. A randomized phase IIIB trial of chemotherapy, bevacizumab, and panitumumab compared with chemotherapy and bevacizumab alone for metastatic colorectal cancer. *J Clin Oncol* 2009;**27**:672–80.
36. Therkildsen C, Bergmann TK, Henrichsen-Schnack T *et al*. The predictive value of KRAS, NRAS, BRAF, PIK3CA and PTEN for anti-EGFR treatment in metastatic colorectal cancer: a systematic review and meta-analysis. *Acta Oncol* 2014; **53**:852–64.
37. Di Nicolantonio F, Martini M, Molinari F *et al*. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. *J Clin Oncol* 2008;**26**: 5705–12.
38. Corcoran RB, Dias-Santagata D, Bergethon K *et al*. BRAF gene amplification can promote acquired resistance to MEK inhibitors in cancer cells harboring the BRAF V600E mutation. *Sci Signal* 2010;**3**:ra84.
39. Klempner SJ, Gershenhorn B, Tran P *et al*. BRAFV600E mutations in high-grade colorectal neuroendocrine tumors may predict responsiveness to BRAF-MEK combination therapy. *Cancer Discov* 2016;**6**:594–600.
40. Moroni M, Veronese S, Benvenuti S *et al*. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to antiEGFR treatment in colorectal cancer: a cohort study. *Lancet Oncol* 2005;**6**:279–86.
41. Lievre A, Bachet JB, Le Corre D *et al*. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res* 2006;**66**:3992–5.
42. De Roock W, Piessevaux H, De Schutter J *et al*. KRAS wild-type state predicts survival and is associated to early radiological response in metastatic colorectal cancer treated with cetuximab. *Ann Oncol* 2008;**19**:508–15.
43. Khambata-Ford S, Garrett CR, Meropol NJ *et al*. Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. *J Clin Oncol* 2007;**25**:3230–7.
44. Lievre A, Laurent-Puig P. Should KRAS mutations be considered an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab? Reply. *J Clin Oncol* 2008;**26**:2601–2.
45. Lievre A, Bachet JB, Boige V *et al*. KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. *J Clin Oncol* 2008;**26**: 374–9.
46. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer. *N Engl J Med* 2009;**361**:98–9.
47. Douillard JY, Siena S, Cassidy J *et al*. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010;**28**:4697–705.
48. Qin S, Li J, Wang L *et al*. Efficacy and tolerability of first-line cetuximab plus leucovorin, fluorouracil, and oxaliplatin (FOLFOX-4) vs FOLFOX-4 in patients with RAS wild-type metastatic colorectal cancer: the open-label, randomized, phase III TAILOR trial. *J Clin Oncol* 2018;**36**:3031–9.
49. Van Cutsem E, Kohne CH, Lang I *et al*. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011;**29**:2011–9.
50. Udar N, Lofton-Day C, Dong J *et al*. Clinical validation of the next-generation sequencing-based Extended RAS Panel assay using metastatic colorectal cancer patient samples from the phase 3 PRIME study. *J Cancer Res Clin Oncol* 2018;**144**: 2001–10.
51. Tveit KM, Guren T, Glimelius B *et al*. Phase III trial of cetuximab with continuous or intermittent fluorouracil, leucovorin, and oxaliplatin (Nordic FLOX) versus FLOX alone in first-line treatment of metastatic colorectal cancer: the NORDIC-VII study. *J Clin Oncol* 2012;**30**:1755–62.
52. Venderbosch S, Nagtegaal ID, Maughan TS *et al*. Mismatch repair status and BRAF mutation status in metastatic colorectal cancer patients: a pooled analysis of the CAIRO, CAIRO2, COIN, and FOCUS studies. *Clin Cancer Res* 2014;**20**: 5322–30.
53. Frattini M, Balestra D, Suardi S *et al*. Different genetic features associated with colon and rectal carcinogenesis. *Clin Cancer Res* 2004;**10**:4015–21.

54. Rajagopalan H, Bardelli A, Lengauer C *et al*. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* 2002;**418**:934.
55. Loupakis F, Ruzzo A, Cremolini C *et al*. KRAS codon 61, 146 and BRAF mutations predict resistance to cetuximab plus irinotecan in KRAS codon 12 and 13 wild-type metastatic colorectal cancer. *Br J Cancer* 2009;**101**:715–21.
56. Bokemeyer C, Van Cutsem E, Rougier P *et al*. Addition of cetuximab to chemotherapy as first-line treatment for KRAS wild-type metastatic colorectal cancer: pooled analysis of the CRYSTAL and OPUS randomised clinical trials. *Eur J Cancer* 2012;**48**:1466–75.
57. Seymour MT, Brown SR, Middleton G *et al*. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol* 2013;**14**:749–59.
58. Saltz LB, Meropol NJ, Loehrer PJ Sr. *et al*. Phase II trial of cetuximab in patients with refractory colorectal cancer that expresses the epidermal growth factor receptor. *J Clin Oncol* 2004;**22**:1201–8.
59. Van Cutsem E, Peeters M, Siena S *et al*. Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. *J Clin Oncol* 2007;**25**:1658–64.
60. Tian S, Simon I, Moreno V *et al*. A combined oncogenic pathway signature of BRAF, KRAS and PI3KCA mutation improves colorectal cancer classification and cetuximab treatment prediction. *Gut* 2013;**62**:540–9.
61. Perrone F, Lampis A, Orsenigo M *et al*. PI3KCA/PTEN deregulation contributes to impaired responses to cetuximab in metastatic colorectal cancer patients. *Ann Oncol* 2009;**20**:84–90.
62. Laurent-Puig P, Cayre A, Manceau G *et al*. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 2009;**27**:5924–30.
63. Rojas AM, Fuentes G, Rausell A *et al*. The Ras protein superfamily: evolutionary tree and role of conserved amino acids (vol 196, pg 189, 2012). *J Cell Biol* 2012;**196**:545.
64. Vigil D, Cherfils J, Rossman KL *et al*. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat Rev Cancer* 2010;**10**:842–57.
65. Giehl K. Oncogenic Ras in tumour progression and metastasis. *Biol Chem* 2005;**386**:193–205.
66. Cox AD, Der CJ. The dark side of Ras: regulation of apoptosis. *Oncogene* 2003;**22**:8999–9006.
67. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment. *N Engl J Med* 2008;**358**:1160–74.
68. Leevers SJ, Paterson HF, Marshall CJ. Requirement for Ras in Raf activation is overcome by targeting Raf to the plasma membrane. *Nature* 1994;**369**:411–4.
69. Marais R, Light Y, Paterson HF *et al*. Ras recruits Raf-1 to the plasma membrane for activation by tyrosine phosphorylation. *EMBO J* 1995;**14**:3136–45.
70. Montagut C, Settleman J. Targeting the RAF-MEK-ERK pathway in cancer therapy. *Cancer Lett* 2009;**283**:125–34.
71. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F *et al*. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res* 2007;**67**:2643–8.
72. Downward J. Targeting RAS signalling pathways in cancer therapy. *Nat Rev Cancer* 2003;**3**:11–22.
73. Rodriguez-Viciana P, Warne PH, Dhand R *et al*. Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 1994;**370**:527–32.
74. Pacold ME, Suire S, Perisic O *et al*. Crystal structure and functional analysis of Ras binding to its effector phosphoinositide 3-kinase gamma. *Cell* 2000;**103**:931–43.
75. Knickelbein K, Zhang L. Mutant KRAS as a critical determinant of the therapeutic response of colorectal cancer. *Genes Dis* 2015;**2**:4–12.
76. Gibbs JB, Sigal IS, Poe M *et al*. Intrinsic GTPase activity distinguishes normal and oncogenic ras p21 molecules. *Proc Natl Acad Sci USA* 1984;**81**:5704–8.
77. Sweet RW, Yokoyama S, Kamata T *et al*. The product of ras is a GTPase and the T24 oncogenic mutant is deficient in this activity. *Nature* 1984;**311**:273–5.
78. Bos JL. ras oncogenes in human cancer: a review. *Cancer Res* 1989;**49**:4682–9.
79. Amado RG, Wolf M, Peeters M *et al*. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;**26**:1626–34.
80. Park JH, Han SW, Oh DY *et al*. Analysis of KRAS, BRAF, PTEN, IGF1R, EGFR intron 1 CA status in both primary tumors and paired metastases in determining benefit from cetuximab therapy in colon cancer. *Cancer Chemother Pharmacol* 2011;**68**:1045–55.
81. Little AS, Balmanno K, Sale MJ *et al*. Amplification of the driving oncogene, KRAS or BRAF, underpins acquired resistance to MEK1/2 inhibitors in colorectal cancer cells (vol 4, art no ra17, 2011). *Sci Signal* 2011;**4**:ra17.
82. Bardelli A, Corso S, Bertotti A *et al*. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer Discov* 2013;**3**:658–73.
83. Valtorta E, Misale S, Sartore-Bianchi A *et al*. KRAS gene amplification in colorectal cancer and impact on response to EGFR-targeted therapy. *Int J Cancer* 2013;**133**:1259–65.
84. Luraghi P, Reato G, Cipriano E *et al*. MET signaling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. *Cancer Res* 2014;**74**:1857–69.
85. Cushman SM, Jiang C, Hatch AJ *et al*. Gene expression markers of efficacy and resistance to cetuximab treatment in metastatic colorectal cancer: results from CALGB 80203 (Alliance). *Clin Cancer Res* 2015;**21**:1078–86.
86. Kawakami H, Okamoto I, Yonesaka K *et al*. The anti-HER3 antibody patritumab abrogates cetuximab resistance mediated by heregulin in colorectal cancer cells. *Oncotarget* 2014;**5**:11847–56.
87. Huang S, Li C, Armstrong EA *et al*. Dual targeting of EGFR and HER3 with MEHD7945A overcomes acquired resistance to EGFR inhibitors and radiation. *Cancer Res* 2013;**73**:824–33.
88. Misale S, Yaeger R, Hobor S *et al*. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012;**486**:532–6.
89. Bettegowda C, Sausen M, Leary RJ *et al*. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med* 2014;**6**:224ra24.
90. Arena S, Bellosillo B, Siravegna G *et al*. Emergence of multiple EGFR extracellular mutations during cetuximab treatment in colorectal cancer. *Clin Cancer Res* 2015;**21**:2157–66.
91. Diaz LA Jr, Williams RT, Wu J *et al*. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. *Nature* 2012;**486**:537–40.

92. Rooney AP. Mechanisms underlying the evolution and maintenance of functionally heterogeneous 18S rRNA genes in Apicomplexans. *Mol Biol Evol* 2004;**21**:1704–11.
93. Montagut C, Dalmases A, Bellosillo B *et al*. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 2012;**18**:221–3.
94. Dong Q, Shi B, Zhou M *et al*. Growth suppression of colorectal cancer expressing S492R EGFR by monoclonal antibody CH12. *Front Med* 2019;**13**:83–93.
95. Esposito C, Rachiglio AM, La Porta ML *et al*. The S492R EGFR ectodomain mutation is never detected in KRAS wild-type colorectal carcinoma before exposure to EGFR monoclonal antibodies. *Cancer Biol Ther* 2013;**14**:1143–6.
96. Yonesaka K, Zejnullahu K, Okamoto I *et al*. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. *Sci Transl Med* 2011;**3**:99ra86.
97. Takegawa N, Yonesaka K, Sakai K *et al*. HER2 genomic amplification in circulating tumor DNA from patients with cetuximab-resistant colorectal cancer. *Oncotarget* 2016;**7**:3453–60.
98. Corcoran RB, Ebi H, Turke AB *et al*. EGFR-mediated reactivation of MAPK signaling contributes to insensitivity of BRAF-mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2012;**2**:227–35.
99. Chapman PB, Hauschild A, Robert C *et al*. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011;**364**:2507–16.
100. Hyman DM, Puzanov I, Subbiah V *et al*. Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med* 2015;**373**:726–36.
101. Roth AD, Tejpar S, Delorenzi M *et al*. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 Trial. *J Clin Oncol* 2010;**28**:466–74.
102. Yang H, Higgins B, Kolinsky K *et al*. Antitumor activity of BRAF inhibitor vemurafenib in preclinical models of BRAF-mutant colorectal cancer. *Cancer Res* 2012;**72**:779–89.
103. Wu Z, Huang M, Gong Y *et al*. BRAF and EGFR inhibitors synergize to increase cytotoxic effects and decrease stem cell capacities in BRAF(V600E)-mutant colorectal cancer cells. *Acta Biochim Biophys Sin* 2018;**50**:355–61.
104. Strickler JH, Wu C, Bekaii-Saab T. Targeting BRAF in metastatic colorectal cancer: maximizing molecular approaches. *Cancer Treatment Rev* 2017;**60**:109–19.
105. Prahallad A, Sun C, Huang SD *et al*. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012;**483**:100–U146.
106. Yaeger R, Cercek A, O'Reilly EM *et al*. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res* 2015;**21**:1313–20.
107. Corcoran RB, Andre T, Atreya CE *et al*. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. *Cancer Discov* 2018;**8**:428–43.
108. Tabernero J, van Geel R, Bendell JC *et al*. Phase I study of the selective BRAFV600 inhibitor encorafenib (LGX818) combined with cetuximab and with or without the a-specific PI3K inhibitor alpelisib (BYL719) in patients with advanced BRAF mutant colorectal cancer. *Eur J Cancer* 2014;**50**:199.
109. Kopetz S, Desai J, Chan E *et al*. Phase II pilot study of vemurafenib in patients with metastatic BRAF-mutated colorectal cancer. *J Clin Oncol* 2015;**33**:4032–8.
110. Flaherty KT, Infante JR, Daud A *et al*. Combined BRAF and MEK inhibition in melanoma with BRAF V600 mutations. *N Engl J Med* 2012;**367**:1694–703.
111. Long GV, Stroyakovskiy D, Gogas H *et al*. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet* 2015;**386**:444–51.
112. Long GV, Grob JJ, Nathan P *et al*. Factors predictive of response, disease progression, and overall survival after dabrafenib and trametinib combination treatment: a pooled analysis of individual patient data from randomised trials. *Lancet Oncol* 2016;**17**:1743–54.
113. Planchard D, Smit EF, Groen HJM *et al*. Dabrafenib plus trametinib in patients with previously untreated BRAF(V600E)-mutant metastatic non-small-cell lung cancer: an open-label, phase 2 trial. *Lancet Oncol* 2017;**18**:1307–16.
114. Planchard D, Besse B, Groen HJM *et al*. Dabrafenib plus trametinib in patients with previously treated BRAF(V600E)-mutant metastatic non-small cell lung cancer: an open-label, multicentre phase 2 trial. *Lancet Oncol* 2016;**17**:984–93.
115. Corcoran RB, Atreya CE, Falchook GS *et al*. Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600-mutant colorectal cancer. *J Clin Oncol* 2015;**33**:4023–31.
116. Long GV, Flaherty KT, Stroyakovskiy D *et al*. Dabrafenib plus trametinib versus dabrafenib monotherapy in patients with metastatic BRAF V600E/K-mutant melanoma: long-term survival and safety analysis of a phase 3 study. *Ann Oncol* 2019;**30**:1848.
117. Corcoran RB, Andre T, Yoshino T *et al*. Efficacy and circulating tumor DNA (ctDNA) analysis of the BRAF inhibitor dabrafenib (D), MEK inhibitor trametinib (T), and anti-EGFR antibody panitumumab (P) in patients (pts) with BRAF V600E-mutated (BRAFm) metastatic colorectal cancer (mCRC). *Ann Oncol* 2016;**27**:
118. Van Cutsem E, Cuyle P, Huijberts S *et al*. BEACON CRC study safety lead-in: Assessment of the BRAF inhibitor encorafenib plus MEK inhibitor binimetinib plus anti-epidermal growth factor receptor antibody cetuximab for BRAFV600E metastatic colorectal cancer. *Ann Oncol* 2018;**29**(suppl_5).
119. Hatzivassiliou G, Liu B, O'Brien C *et al*. ERK inhibition overcomes acquired resistance to MEK inhibitors. *Mol Cancer Ther* 2012;**11**:1143–54.
120. Morris EJ, Jha S, Restaino CR *et al*. Discovery of a novel ERK inhibitor with activity in models of acquired resistance to BRAF and MEK inhibitors. *Cancer Discov* 2013;**3**:742–50.
121. Miyamoto Y, Suyama K, Baba H. Recent advances in targeting the EGFR signaling pathway for the treatment of metastatic colorectal cancer. *Int J Mol Sci* 2017;**18**:752.
122. Porru M, Pompili L, Caruso C *et al*. Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities. *J Exp Clin Cancer Res* 2018;**37**:57.
123. Liu P, Wang Y, Li X. Targeting the untargetable KRAS in cancer therapy. *Acta Pharm Sin B* 2019;**9**:871–9.
124. Bahrami A, Hassanian SM, ShahidSales S *et al*. Targeting RAS signaling pathway as a potential therapeutic target in the treatment of colorectal cancer. *J Cell Physiol* 2018;**233**:2058–66.
125. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. *Oncogene* 2008;**27**:5497–510.
126. E J, Xing J, Gong H *et al*. Combine MEK inhibition with PI3K/mTOR inhibition exert inhibitory tumor growth effect on KRAS and PIK3CA mutation CRC xenografts due to reduced expression of VEGF and matrix metallopeptidase-9. *Tumor Biol* 2015;**36**:1091–7.
127. Ostrem JM, Peters U, Sos ML *et al*. K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013;**503**:548–51.

128. Lito P, Solomon M, Li LS *et al*. Allele-specific inhibitors inactivate mutant KRAS G12C by a trapping mechanism. *Science* 2016;**351**:604–8.
129. Shipman L. Signalling: putting the brakes on KRAS-G12C nucleotide cycling. *Nat Rev Cancer* 2016;**16**:127.
130. Canon J, Rex K, Saiki AY *et al*. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 2019;**575**: 217–23.
131. Balasubramanian S, Hurley LH, Neidle S. Targeting G-quadruplexes in gene promoters: a novel anticancer strategy? *Nat Rev Drug Discov* 2011;**10**:261–75.
132. Chambers VS, Marsico G, Boutell JM *et al*. High-throughput sequencing of DNA G-quadruplex structures in the human genome. *Nat Biotechnol* 2015;**33**:877–81.
133. Cogoi S, Xodo LE. G4 DNA in ras genes and its potential in cancer therapy. *Biochim Biophys Acta* 2016;**1859**:663–74.
134. Morgan RK, Batra H, Gaerig VC *et al*. Identification and characterization of a new G-quadruplex forming region within the kRAS promoter as a transcriptional regulator. *Biochim Biophys Acta* 2016;**1859**:235–45.
135. Porru M, Artuso S, Salvati E *et al*. Targeting G-quadruplex DNA structures by EMICORON has a strong antitumor efficacy against advanced models of human colon cancer. *Mol Cancer Ther* 2015;**14**:2541–51.
136. Porru M, Zizza P, Franceschin M *et al*. EMICORON: a multitargeting G4 ligand with a promising preclinical profile. *Biochim Biophys Acta* 2017;**1861**:1362–70.
137. Cox AD, Der CJ, Philips MR. Targeting RAS membrane association: back to the future for anti-RAS drug discovery? *Clin Cancer Res* 2015;**21**:1819–27.
138. Chandra A, Grecco HE, Pisupati V *et al*. The GDI-like solubilizing factor PDEdelta sustains the spatial organization and signalling of Ras family proteins. *Nat Cell Biol* 2012;**14**:148–58.
139. Zimmermann G, Papke B, Ismail S *et al*. Small molecule inhibition of the KRAS-PDEdelta interaction impairs oncogenic KRAS signalling. *Nature* 2013;**497**:638–42.
140. Papke B, Murarka S, Vogel HA *et al*. Identification of pyrazolopyridazinones as PDEdelta inhibitors. *Nat Commun* 2016;**7**: 11360.
141. Mayers JR, Torrence ME, Danai LV *et al*. Tissue of origin dictates branched-chain amino acid metabolism in mutant Kras-driven cancers. *Science* 2016;**353**:1161–5.
142. Bryant KL, Mancias JD, Kimmelman AC *et al*. KRAS: feeding pancreatic cancer proliferation. *Trends Biochem Sci* 2014;**39**: 91–100.
143. Hutton JE, Wang X, Zimmerman LJ *et al*. Oncogenic KRAS and BRAF drive metabolic reprogramming in colorectal cancer. *Mol Cell Proteomics* 2016;**15**:2924–38.
144. Song M, Kim SH, Im CY *et al*. Recent development of small molecule glutaminase inhibitors. *Curr Topics Med Chem* 2018; **18**:432–43.
145. Yun J, Mullarky E, Lu C *et al*. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* 2015;**350**:1391–6.
146. Champiat S, Dercle L, Ammari S *et al*. Hyperprogressive disease is a new pattern of progression in cancer patients treated by anti-PD-1/PD-L1. *Clinical Cancer Res* 2017;**23**:1920–8.
147. Dong ZY, Zhong WZ, Zhang XC *et al*. Potential predictive value of TP53 and KRAS mutation status for response to PD-1 blockade immunotherapy in lung adenocarcinoma. *Clin Cancer Res* 2017;**23**:3012–24.
148. Jeanson A, Tomasini P, Souquet-Bressand M *et al*. Efficacy of immune checkpoint inhibitors in KRAS-mutant non-small cell lung cancer (NSCLC). *J Thorac Oncol* 2019;**14**:1095–101.
149. Le DT, Uram JN, Wang H *et al*. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015;**372**:2509–20.
150. Chan DA, Giaccia AJ. Harnessing synthetic lethal interactions in anticancer drug discovery. *Nat Rev Drug Discov* 2011; **10**:351–64.
151. Dietlein F, Kalb B, Jokic M *et al*. A synergistic interaction between Chk1- and MK2 inhibitors in KRAS-mutant cancer. *Cell* 2015;**162**:146–59.
152. Aguirre AJ, Hahn WC. Synthetic lethal vulnerabilities in KRAS-mutant cancers. *Cold Spring Harb Perspect Med* 2018;**8**:a031518.
153. Corcoran RB, Cheng KA, Hata AN *et al*. Synthetic lethal interaction of combined BCL-XL and MEK inhibition promotes tumor regressions in KRAS mutant cancer models. *Cancer Cell* 2013;**23**:121–8.
154. Seca AML, Pinto D. Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. *Int J Mol Sci* 2018;**19**:263.
155. Kunnumakkara AB, Bordoloi D, Padmavathi G *et al*. Curcumin, the golden nutraceutical: multitargeting for multiple chronic diseases. *Br J Pharmacol* 2017;**174**:1325–48.
156. Dhillon S. Regorafenib: a review in metastatic colorectal cancer. *Drugs* 2018;**78**:1133–44.
157. Grothey A, Van Cutsem E, Sobrero A *et al*. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013;**381**:303–12.
158. Li J, Qin SK, Xu RH *et al*. Regorafenib plus best supportive care versus placebo plus best supportive care in Asian patients with previously treated metastatic colorectal cancer (CONCUR): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2015;**16**:619–29.
159. Wu CS, Wu SY, Chen HC *et al*. Curcumin functions as a MEK inhibitor to induce a synthetic lethal effect on KRAS mutant colorectal cancer cells receiving targeted drug regorafenib. *J Nutr Biochem* 2019;**74**:108227.
160. Lamba S, Russo M, Sun C *et al*. RAF suppression synergizes with MEK inhibition in KRAS mutant cancer cells. *Cell Rep* 2014;**8**:1475–83.