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REVIEW ARTICLE

Targeting EGFR and RAS/RAF Signaling in the Treatment of Metastatic Colorectal Cancer: From Current Treatment Strategies to Future Perspectives

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Abstract
The epidermal growth factor receptor (EGFR) and RAS/RAF signaling pathway plays pivotal roles in tumor progression via proliferation, survival, invasion, and immune evasion. Two anti-EGFR monoclonal antibodies, cetuximab and panitumumab, have become essential components in the treatment of patients with metastatic colorectal cancer (mCRC). Treatment with these anti-EGFR antibodies has shown definite benefits when administered in all treatment lines and is strongly recommended as the preferred regimen to prolong survival, especially when administered in the first- and third-lines. Recent efforts have revealed not only mechanisms responsible for resistance to anti-EGFR antibodies, including expanded *RAS* mutations as a negative predictive biomarker, but also the possibility of continuing anti-EGFR antibody treatment in combination with chemotherapy. Furthermore, the challenges associated with the pharmaceutical development of treatments for patients with mutant-type *BRAF* mCRC are ongoing. In this review, we provide an overview of the EGFR and RAS/RAF signaling pathway and antitumor activity, focusing on practical aspects such as established treatments including patient selection, treatment strategies, and future perspectives for drug development targeting the EGFR and RAS/RAF signaling pathway.

Key Points

The anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies cetuximab and panitumumab prolong the survival of patients with metastatic colorectal carcinoma (mCRC).

Small molecule inhibitors to activated BRAF present new treatment options in *BRAF* mutated mCRC patients.

Optimizing treatment by integrating these targeted drugs with anti-vascular endothelial growth factor (anti-VEGF) therapy and chemotherapy and finding the best sequencing approaches are current challenges in this area.

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1 Introduction

Colorectal cancer (CRC) is one of the most frequently occurring cancers worldwide, and metastatic CRC (mCRC) continues to be associated with a poor prognosis [1]. Treatment strategies for mCRC patients are limited, but recent efforts to improve survival outcomes among mCRC patients have focused on the combination of conventional chemotherapies with agents targeting biological pathways that are pivotal to cancer pathogenesis. The epidermal growth factor receptor (EGFR) family plays a key role in tumor progression via proliferation, survival, invasion, and immune evasion [2]. Two anti-EGFR monoclonal antibodies, cetuximab and panitumumab, are now approved for the treatment of mCRC worldwide. Furthermore, the benefits of anti-EGFR antibodies have been confirmed to be limited to patients whose tumors do not harbor mutations in retrovirus-associated DNA sequences (*RAS*) genes, including *KRAS* and *NRAS*; these genes encode proteins downstream of EGFR, in the RAS/v-RAF 1 murine leukemia viral oncogene homolog 1 (RAF)/mitogen-activated protein kinase (MAPK) pathway [2–8]. *BRAF* and *PIK3CA* mutations have also been reported as potential predictive biomarkers of the efficacies of these

anti-EGFR antibodies [9–13]. Nevertheless, chemotherapy for mCRC patients who harbor *BRAF* mutant tumors and have a poor prognosis remains insufficient.

In this review, we provide an overview of EGFR and RAS/RAF signaling and antitumor activity of agents that target these pathways, focusing on practical aspects such as established treatments including patient selection, treatment strategies, and future perspectives for drug development targeting the EGFR and RAS/RAF signaling pathway.

2 Clinical Trials Establishing the Use of Anti-EGFR Therapies for mCRC

The anti-EGFR monoclonal antibodies cetuximab and panitumumab were approved for the treatment of mCRC patients in the 2000s. In a phase II study (BOND trial) comparing cetuximab plus irinotecan with cetuximab monotherapy to verify the assumption that cetuximab circumvents irinotecan resistance, cetuximab plus irinotecan improved the response rate (RR) and progression-free survival (PFS) in mCRC patients who were refractory to irinotecan monotherapy [14]. Cetuximab was initially approved based on these results. Subsequently, the NCIC CTG-CO.17 trial comparing cetuximab plus best supportive care (BSC) and BSC alone in EGFR-positive refractory mCRC patients also showed a survival benefit with cetuximab [15].

In the second-line treatment of mCRC, several clinical trials have demonstrated the value of adding cetuximab or panitumumab to conventional chemotherapy in terms of RR and PFS, but unfortunately not in terms of overall survival (OS) [16–19]. It is discussed that these results could be caused by the impact of a crossover design. Regarding the comparison of anti-EGFR antibodies and bevacizumab treatment beyond progression, subsequent anti-EGFR treatment may affect the lack of improvement in OS [20]. Considering these results, anti-EGFR antibodies only improved the RR compared with bevacizumab beyond progression in the second-line treatment of mCRC patients harboring wild-type *KRAS*.

In a first-line setting, a phase III trial (CRYSTAL trial) comparing FOLFIRI either alone or in combination with cetuximab in patients with EGFR-positive mCRC demonstrated that combination with cetuximab reduced the risk of mCRC progression (8.9 vs. 8.0 months; hazard ratio [HR] 0.85; 95% confidence interval [CI] 0.72–0.99; $P=0.048$) [3]. The value of adding anti-EGFR antibodies to oxaliplatin-based chemotherapy was investigated in three pivotal trials: OPUS, COIN, and PRIME. In the phase II OPUS trial, a significantly higher RR was observed for the FOLFOX4 plus cetuximab arm (46% vs. 36%, $P=0.0064$), but PFS or OS did not improve compared with the FOLFOX4 arm [4]. Also, the phase III COIN trial did not confirm a survival benefit for the addition of cetuximab to oxaliplatin-based chemotherapy (FOLFOX or CAPOX) in the first-line treatment of patients with mCRC (17.0 vs. 17.9 months; HR 1.04; 95% CI 0.87–1.23; $P=0.67$), even though a higher RR was observed for cetuximab plus oxaliplatin-based chemotherapy (64% vs. 57%, $P=0.049$) [21]. The unexpected result of the COIN trial is likely to be attributed to the specific skin toxic effect of the CAPOX regimen when cetuximab was added. Similar results were observed in another phase III trial [22]. Regarding panitumumab, the phase III PRIME trial demonstrated the value of adding panitumumab to FOLFOX4 in terms of PFS (9.6 vs. 8.0 months; HR 0.80; 95% CI 0.66–0.97; $P=0.02$) in patients with wild-type *KRAS* mCRC, but not in terms of OS (23.9 vs. 19.7 months; HR 0.83; 95% CI 0.67–1.02; $P=0.072$) [23]. Nowadays, the efficacy of anti-EGFR antibodies is known to differ according to tumor biology. As described below, retrospective subgroup analyses of the CRYSTAL, OPUS, and PRIME trials have revealed that the benefit of anti-EGFR antibodies is only obvious in a molecularly selected population [24–26]. A prospective trial that did select for wild-type *RAS* mCRC patients, the phase III TAILOR trial, demonstrated a survival benefit for adding cetuximab to FOLFOX4 in the first-line treatment of patients with wild-type *RAS* mCRC (PFS: HR 0.69; 95% CI 0.54–0.89; $P=0.004$; OS: HR 0.76; 95% CI 0.61–0.96; $P<0.001$), confirming the value of adding anti-EGFR antibodies to oxaliplatin-based chemotherapy [27].

Further, the efficacy of maintenance therapy with anti-EGFR antibodies has been evaluated in several trials. In the phase II MACRO-II trial, single-agent cetuximab following mFOLFOX6 plus cetuximab achieved non-inferiority in terms of PFS at 9 months, with fewer adverse events compared with continuous mFOLFOX6 plus cetuximab (60% vs. 72%, non-inferiority $P=0.25$) [28]. A retrospective analysis of the PRIME and PEAK trials also showed that maintenance with panitumumab plus 5-FU/LV after discontinuation of oxaliplatin was well tolerated, and PFS and OS were numerically longer compared to maintenance with panitumumab alone [29]. In the VALENTINO trial, maintenance therapy with single-agent panitumumab following mFOLFOX6 plus panitumumab had a significantly shorter PFS than that with 5-FU/LV plus panitumumab (HR 1.55; 95% CI 1.09–2.20; $P=0.011$), and sparing of 5-FU/LV did not contribute to reducing the toxicity [30]. Considering these results, 5-FU/LV plus an anti-EGFR antibody is one of the preferred maintenance regimens using anti-EGFR antibodies, and further investigation comparing it with maintenance therapy with bevacizumab is needed.

3 Development of Predictive Biomarkers in Targeting EGFR

The EGFR and downstream components of the pathway have a key role in tumorigenesis via the regulation of proliferation, angiogenesis and metastasis, and cetuximab and

panitumumab bind specifically to both EGFR homodimers and its heterodimers [2, 31–33]. In the past few decades, these components of the EGFR and RAS/RAF signaling pathway have been systematically evaluated for their value as predictive biomarkers of EGFR therapies. Although other mechanisms exhibiting resistance to anti-EGFR antibodies, such as *HER2/MET* amplification and *PIK3CA* mutation, are important, we will mainly focus on the predictive value of biomarkers involved in the EGFR and RAS/RAF pathway [12, 34] (Fig. 1).

3.1 EGFR and Its Ligands

EGFR overexpression as revealed by immunohistochemistry (IHC) has led to initial clinical trials investigating patient selection for cetuximab or panitumumab with promising results [14, 35]. However, several studies have demonstrated that patients can benefit from the addition of cetuximab in the absence of a positive EGFR IHC result [14, 23, 36–38]. Although EGFR alterations including gene overexpression or gene copy number alteration were also expected to be predictive biomarkers for anti-EGFR antibodies, no unified view has been obtained so far [39, 40]. A single point mutation in the ectodomain of *EGFR* S468R confers acquired or secondary resistance in mCRC treated with cetuximab, although this mechanism is not observed in mCRC treated with panitumumab [41]. This mutation was not detected in wild-type *KRAS* mCRC before exposure to anti-EGFR antibodies and is not considered to be a mechanism of primary resistance to cetuximab [42]. Regarding EGFR ligands such as epiregulin (EREG) and amphiregulin (AREG), several reports have shown that the gene expression of EGFR ligands in primary tumors significantly predicts the outcomes of patients treated with anti-EGFR antibodies, especially for those with wild-type *KRAS* mCRC [43–46]. Another post hoc analysis of the phase III AIO KRK-0207 trial demonstrated the prognostic value of high-expression levels of *AREG* and *EREG* in patients undergoing first-line chemotherapy including oxaliplatin, fluoropyrimidine, and bevacizumab [47]. However, further investigation evaluating optimal unified testing methods and cut-offs is needed.

3.2 *RAS* Mutations

The RAS GTPase is a master signaling protein at the hub of numerous signal transduction pathways (including the EGFR pathway) and interacts with many effector proteins to regulate cell proliferation, survival, migration, and

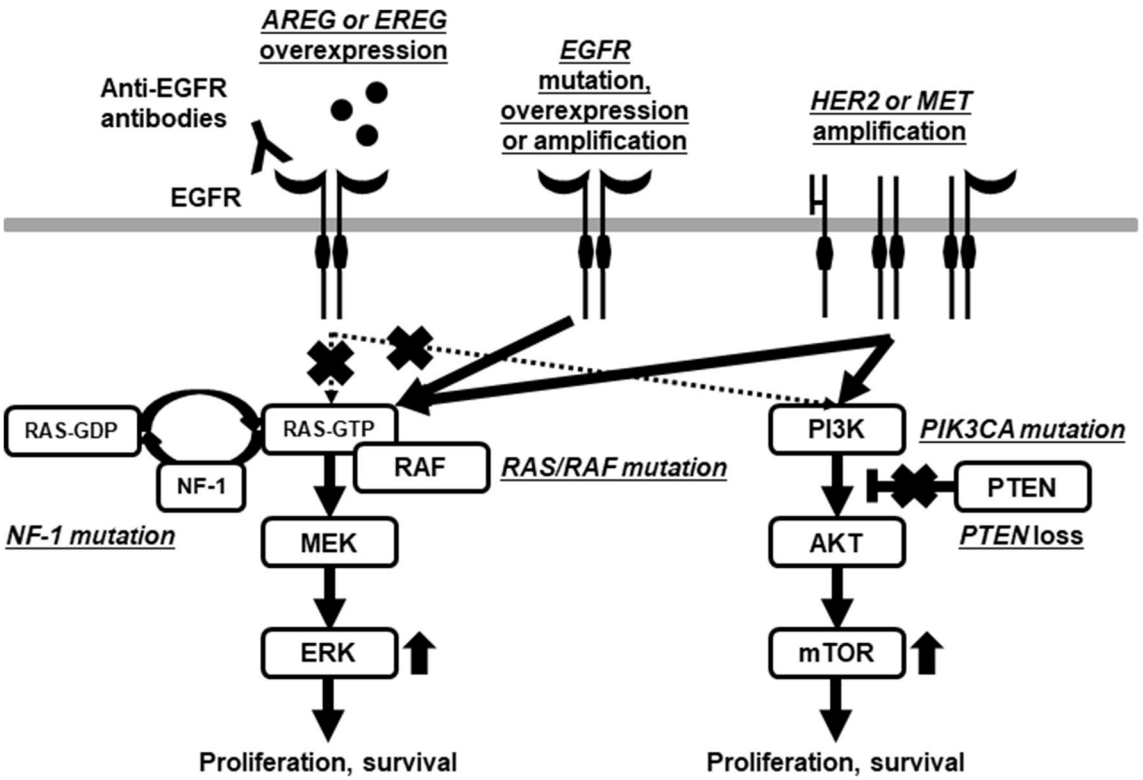

Fig. 1 Mechanisms of resistance to anti-EGFR antibodies. Aberrated gene alterations, including *EGFR*, *AREG*, *EREG*, *NF-1*, *RAS*, *BRAF*, *PIK3CA*, and *EGFR* S492R mutations, *PTEN* loss, and *HER2/MET* amplification, contribute to resistance via the activation of EGFR downstream components, regardless of EGFR blockade. Each mechanism is underlined and noted. *EGFR* epidermal growth factor receptor

apoptosis [48]. The three major isoforms of RAS (KRAS, NRAS, and HRAS) are mutated in around 45% of mCRC, primarily at the active site at residues G12, G13, and Q61 near the γ-phosphate of the guanosine triphosphate substrate [49–51]. The benefit of anti-EGFR antibodies is limited in these cases, with up to 65% of mCRC patients with *KRAS* mutations in exon 2 in codon 12/13 being resistant to these treatments [2, 7, 8]. Recently, multiple studies have revealed that other mutations in *KRAS* exons 3 and 4 or *NRAS* exons 2 to 4 can also predict a lack of benefit from anti-EGFR antibodies, as summarized in Table 1 [26]. Based on these results, in the 2010s, the indications for the use of anti-EGFR antibodies were extended from wild-type *KRAS* to all wild-type *RAS* tumors [52–54].

This change has led to a reassessment of the value of adding anti-EGFR antibodies in a second-line setting. In a phase III trial (20050181 trial) to evaluate the effect of the addition of panitumumab to FOLFIRI chemotherapy, the HR of the PFS for panitumumab plus FOLFIRI versus FOLFIRI favored panitumumab more strongly in a wild-type *RAS* population than in a wild-type *KRAS* exon 2 population (HR 0.70; 95% CI 0.54–0.91; $P=0.007$) [55]. Another subanalysis of a phase II trial comparing panitumumab plus FOLFIRI with bevacizumab plus FOLFIRI in a second-line setting for wild-type *KRAS* exon 2 mCRC (WJOG6210G

Table 1 Comparison of clinical studies of anti-EGFR antibody according to *RAS* status

Trial name	Regimen	*N*		RR (%)		PFS (months)		OS (months)	
		RAS wt	*RAS* mt	*RAS* wt	*RAS* mt	*RAS* wt	*RAS* mt	*RAS* wt	*RAS* mt
OPUS	FOLFOX4	49	75	29	51	5.8	7.8	17.8	17.8
	FOLFOX4 + cetu	38	92	58	37	12.0	5.6	19.8	13.5
						HR 0.53	HR 1.54	HR 0.94	HR 1.29
						$P=0.062$	$P=0.031$	$P=0.80$	$P=0.16$
CRYSTAL	FOLFIRI	189	214	38.6	36.0	8.4	7.5	20.2	17.7
	FOLFIRI + cetu	178	246	66.3	31.7	11.4	7.4	28.4	16.4
						HR 0.56	HR 1.10	HR 0.69	HR 1.05
						$P=0.0002$	$P=0.47$	$P=0.002$	$P=0.64$
PRIME	FOLFOX4	253	276	NA	NA	7.9	8.7	20.2	19.2
	FOLFOX4 + pani	259	272	NA	NA	10.1	7.3	26.0	15.6
						HR 0.72	HR 1.31	HR 0.78	HR 1.25
						$P=0.004$	$P=0.14$	$P=0.04$	$P=0.034$
20050181	FOLFIRI	211	294	10	13	4.4	4.0	13.9	11.1
	FOLFIRI + pani	204	299	41	15	6.4	4.8	16.2	11.8
						HR 0.70	HR 0.86	HR 0.80	HR 0.91
						$P=0.006$	$P=0.14$	$P=0.08$	$P=0.34$
20020408	BSC	63	144	0	0	7 weeks	7.3 weeks	NA	NA
	BSC + pani	73	99	16	1	14.1 weeks	7.4 weeks	NA	NA
						HR 0.36	HR 0.97		
						$P<0.0001$	$P=0.73$		
PEAK	mFOLFOX6 + bev	82	27	54	NA	10.1	8.9	28.9	NA
	mFOLFOX6 + pani	88	24	58	NA	13.0	7.8	41.3	NA
						HR 0.66	HR 1.39	HR 0.63	
						$P=0.03$	$P=0.32$	$P=0.06$	
FIRE-3	FOLFIRI + bev	171	86	59.6	51.2	10.2	10.1	25.6	20.6
	FOLFIRI + cetu	171	92	65.5	38.0	10.4	7.5	33.1	20.9
						HR 0.93	HR 1.31	HR 0.70	HR 1.09
						$P=0.54$	$P=0.085$	$P=0.011$	$P=0.60$
CALGB80405	FOLFOX/IRI + bev	256	42	53.8	NA	11.3	NA	31.2	22.3
	FOLFOX/IRI + cetu	270	53	68.6	NA	11.4	NA	32.0	28.7
						HR 1.1		HR 0.9	HR 0.74
						$P=0.31$		$P=0.40$	$P=0.21$

bev bevacizumab, *BSC* best supportive care, *cetu* cetuximab, *EGFR* epidermal growth factor receptor, *HR* hazard ratio, *mt* mutant-type, *NA* not available, *OS* overall survival, *pani* panitumumab, *PFS* progression-free survival, *RR* response rate, *wt* wild-type

trial) demonstrated a tendency toward a better OS for all wild-type *RAS* tumors in the FOLFIRI plus panitumumab arm (18.9 vs. 16.1 months), resulting in a significant interaction (P for interaction = 0.026) [19]. Furthermore, the RR was 52.5% for FOLFIRI plus panitumumab and 2.6% for FOLFIRI plus bevacizumab ($P < 0.001$). Recently, the role of EGFR therapies in the second-line treatment of wild-type *KRAS* mCRC patients after progression on cetuximab has been explored [56, 57]. In the CAPRI-GOIM trial evaluating the possibility of continuing cetuximab treatment, the PFS for treatment with FOLFOX plus cetuximab, compared with FOLFOX alone, was significantly prolonged in all wild-type *RAS* patients (HR 0.56; 95% CI 0.33–0.94; $P = 0.025$). This result suggests that continuing cetuximab treatment in combination with chemotherapy might confer therapeutic efficacy in all wild-type *RAS* patients, leading to the "re-challenge" issue that will be described later (see Sect. 4.1) [58].

The selection of patients on the basis of their *RAS* status was a notable change in the treatment of mCRC. A remaining issue is whether the antiangiogenic drug bevacizumab or anti-EGFR antibodies are the best option for first-line treatment. Pivotal studies, including two phase III trials, the Cancer and Leukemia Group B (CALGB) 80405 trial and the FIRE-3 trial, and the phase II PEAK trial comparing anti-EGFR antibodies and bevacizumab as a first-line treatment for wild-type *KRAS* exon 2 mCRC, have tried to resolve this issue. In the overall wild-type *RAS* population, the FIRE-3 trial and the PEAK trial demonstrated the superiority of anti-EGFR antibodies in terms of OS, RR, depth of response, and early tumor shrinkage [8, 59, 60]. However, the CALGB80405 trial did not show the same superiority of cetuximab in the overall wild-type *RAS* population [61]. Although this discrepancy might have been caused by differences in post-progression treatment and combination regimens, no unified view has been obtained so far. Several meta-analyses of these three randomized clinical trials have demonstrated the superiority of first-line anti-EGFR antibodies in terms of RR and OS, compared with anti-vascular endothelial growth factor (anti-VEGF) therapy, among wild-type *KRAS* and the overall wild-type *RAS* population with mCRC [62, 63]. There seems to be little doubt from these results that EGFR therapies in combination with chemotherapy have a major likelihood of providing an improvement in survival for patients with all wild-type *RAS* mCRC.

Furthermore, CRC is known to exhibit differences in its incidence, pathogenesis, molecular pathways, and outcome depending on the location of the primary tumor [64, 65]. A recent meta-analysis of over a million CRC patients confirmed the prognostic role of tumor sidedness in all stages of disease. A left-sided primary tumor location was significantly associated with a reduced risk of death, compared with a right-sided primary tumor location (HR 0.82; 95% CI 0.79–0.84; $P < 0.001$) [66]. Several retrospective analyses have also demonstrated that the primary tumor location may also be a predictive biomarker of anti-EGFR antibodies [67–74]. Several meta-analyses including randomized first-line studies in patients with mCRC support these results, especially in terms of the value of sidedness as a predictive biomarker of the efficacies of anti-EGFR antibodies [62, 75, 76]. These analyses showed a similar worse prognosis for patients with right-sided tumors, compared with those with left-sided tumors, in patients with wild-type *RAS* mCRC. Moreover, a greater effect of chemotherapy plus anti-EGFR antibody treatment, compared with chemotherapy or chemotherapy plus bevacizumab, was observed in patients with left-sided tumors. For right-sided tumors, there is no significant difference between anti-EGFR and anti-VEGF antibodies in terms of survival in post hoc analyses of the FIRE-3, CALGB80405, and PEAK trials. Nowadays, we understand that chemotherapy plus an anti-EGFR antibody should be the preferred first-line treatment option for patients with mCRC harboring left-sided wild-type *RAS* tumors [52–54].

3.3 *BRAF* Mutations

BRAF is a serine/threonine kinase that is active directly downstream of KRAS and that activates MEK through its phosphorylation in the RAS signaling pathway [77]. The Cancer Genome Atlas identified *BRAF* V600E mutations in several malignancies, including CRC [78]. Metastatic CRC harbors *BRAF* V600E mutations at a frequency of approximately 5–11%, and the *BRAF* V600E mutation is mutually exclusive with *KRAS* mutations [51, 79–81]. CRC harboring *BRAF* V600E mutations is known to be associated with right-sided primary tumors, older women, high-grade tumors, and precursor sessile serrated adenomas [82, 83]. Several post hoc analyses of phase III trials have reported the predictive value of *BRAF* V600E mutations for the efficacy of anti-EGFR antibodies [5, 17, 22, 26, 84–86]. However, the predictive values were not in accordance in all the reports and remain controversial. A meta-analysis of phase III trials and a phase II trial in chemorefractory patients reported that the addition of anti-EGFR antibodies did not significantly improve PFS (HR 0.88; 95% CI 0.67–1.14; $P = 0.33$) or OS (HR 0.91; 95% CI 0.62–1.34, $P = 0.63$) in the *BRAF* mutation subgroup [87]. Furthermore, another similar meta-analysis of eight randomized control trials demonstrated that the HR for the OS benefit with anti-EGFR antibodies was 0.97 (95% CI 0.67–1.41) for *BRAF* mutant tumors and 0.81 (95% CI 0.70–0.95) for *BRAF* wild-type tumors. However, no statistically significant difference was observed between these tumors (interaction $P = 0.43$) [88]. On the other hand, in the FIRE-3 trial, early tumor shrinkage as a strong early on-treatment parameter associated with outcome was identified in a certain number of patients with *BRAF* mutant

tumors treated with FOLFIRI plus cetuximab [13]. In the VOLFI trial, the addition of panitumumab to FOLFOXIRI showed a high RR in *BRAF* mutant mCRC [89]. Considering these results, some patients may receive clinical benefit from treatment with anti-EGFR antibodies even if they are *BRAF* mutant. Collectively, limited data are available supporting the exclusion of patients with *BRAF* mutant mCRC from treatment with anti-EGFR antibodies. Nowadays, it may be suspected that the *BRAF* mutation is not a definitive predictive biomarker for anti-EGFR antibodies but a promising targetable subgroup for combination therapy blocking multiple pathways (see below).

Recently, *BRAF* mutations that do not result in an amino acid substitution at position 600 (*BRAF* non-V600E mutations) have been reported. The incidence of *BRAF* non-V600E mutation is reportedly 1–5% in mCRC, and the mutation's kinase activity can be classified up to a level of activity similar to that of the *BRAF* V600E mutation [56, 90–92]. Unlike the *BRAF* V600E mutation, however, *BRAF* non-V600E mutations are correlated with significantly better survival [93]. A retrospective study reported that the clinical outcomes, including RR, PFS, and OS, were similar between *RAS* mutation, *BRAF* V600E mutation, and certain *BRAF* non-V600E mutations including G469A, L485F, Q524L, L525R, D594G, and V600R; in addition, *BRAF* non-V600E mutations contributed to a smaller benefit from anti-EGFR antibody [94]. On the other hand, there is evidence that *BRAF* non-V600E mutant mCRC can respond to panitumumab [95]. *BRAF* non-V600E mutations including the abovementioned mutations can be classified into three different classes depending on the different extents of dependency on RAS, which may dictate the response to anti-EGFR antibodies [96, 97]. It has been reported that almost half of patients with class 3 *BRAF* non-V600E mutations responded to anti-EGFR therapy, while response was rare for patients with class 2 *BRAF* non-V600E mutations [98]. It may be clinically helpful if BRAF tests focused on not only the *BRAF* V600E mutation but also on non-V600E mutations. Furthermore, the upregulated kinase activity of BRAF and/or alternative signaling through CRAF could lead to the incomplete blockade of the MEK pathway in a preclinical model, and another strategy attempting to inhibit signaling through the MAPK pathway is being developed for these subgroups of *BRAF* V600E and non-V600E mutations [92, 97, 99].

3.4 *NF-1* Alterations

NF-1 is a tumor suppressor gene that encodes a neurofibromin, which functions as a suppressive regulator of the RAS signaling pathway [100]. The frequency of somatic *NF-1* mutations in CRC is reportedly 1–6% [101]. In lung cancer, several studies reported that reduced *NF-1* expression leading to the activation of the MAPK pathway via *NF-1* deletion or mutations was associated with the development of primary and acquired resistance to EGFR tyrosine kinase inhibitors [102, 103]. An analysis of the mutation landscape of 33 Chinese mCRC specimens demonstrated that *NF-1* alterations may be candidates for predictive biomarkers for anti-EGFR antibodies [104]. Furthermore, the co-existence of *NF-1* mutations and *BRAF* mutations leads to the development of resistance to BRAF inhibitors in melanoma cells [105]. Therefore, *NF-1* alterations are expected to be a potential target of combination therapies inhibiting the EGFR and RAS/RAF signaling pathway in mCRC, and further investigation is needed.

4 Challenges in Targeting EGFR and RAS/RAF Signaling

Although optimizing patient selection according to the *RAS* mutation status and the primary tumor location have benefited patients with mCRC harboring wild-type *RAS* tumors, primary or acquired resistance to anti-EGFR antibodies remains an important issue. Over the past decade, many studies have attempted to overcome these resistances by focusing on the EGFR and RAS/RAF signaling pathway.

4.1 Retreatment with Anti-EGFR Antibodies

A recent phase III study has shown that regorafenib and TAS-102 are superior to a placebo in refractory mCRC, although with limited efficacy (RR 1.0% and 1.6%, respectively) [106, 107]. Thus, there is an urgent need to develop more effective later-line treatments for mCRC.

The development of high throughput sequencing technologies has provided not only a highly efficient, rapid, and low-cost DNA sequencing platform, but also the possibility of performing DNA sequencing using "liquid" biopsies. Although tissue biopsy is the standard of care for tumor diagnosis, traditional tumor needle or excisional biopsies from certain metastatic diseases are invasive procedures, and obtaining a sufficient tissue sample can be difficult in patients with mCRC. So far, a liquid biopsy seems to be a promising, minimally invasive technique for diagnosing the current tumor status and monitoring mCRC patients during anti-cancer treatment, with good concordance to tissue specimens [108–112]. Wild-type *RAS* mCRC tumors are well known to develop resistance to anti-EGFR antibodies by acquiring gene mutations, including *RAS*, that enable cells to escape from the ongoing treatment, but limited data are available regarding the reversibility of their mutation statuses [34, 113, 114]. There is a hypothesis that the occurrence of disease progression after an initial response in wild-type *RAS* mCRC may potentially

contribute to the progressive prevalence of a mutated clone caused by the acquisition of resistance to the anti-EGFR antibody during therapy. To verify this, several phase II studies have examined re-challenge treatments with anti-EGFR antibodies in patients with wild-type *RAS* mCRC at the time of diagnosis (Table 2) [115–120]. The CRICKET and E-Rechallenge trials demonstrated a tendency toward a higher RR, compared with other trials. A retrospective study analyzing 89 mCRC patients who received cetuximab or cetuximab plus erlotinib also indicated that a response while receiving prior anti-EGFR antibody and a longer interval length between anti-EGFR therapies may be associated with the efficacy of re-challenge treatment with anti-EGFR antibody [121]. An analysis of the post-progression circulating tumor DNA (ctDNA) profiles of patients with wild-type *RAS/BRAF* mCRC treated with anti-EGFR antibodies who acquired *RAS* and/or *EGFR* mutations during therapy supports this hypothesis [122]. This analysis demonstrated that the cumulative half-life of the *RAS* and *EGFR* relative mutant allele frequency was 4.4 months, and patients had a higher RR during re-challenge therapies after longer time intervals. Moreover, the CRICKET trial demonstrated that the PFS was longer in patients with wild-type *RAS* mCRC in ctDNA at the re-challenge baseline (HR 0.44; 95% CI 0.18–0.98; $P = 0.026$) [119]. Although these results suggest the contribution of the interval length between anti-EGFR antibody therapies in overcoming the acquired resistance to anti-EGFR antibody, an ongoing phase III trial (FIRE-4, NCT02934529) should provide further useful indications. The FIRE-4 trial has a planned enrolment of 550 patients with wild-type *RAS* mCRC who will receive first-line cetuximab-based therapy and third-line cetuximab as a re-challenge treatment and will include *RAS* assessment using liquid biopsies to assess progressive disease.

4.2 Development of Combination Therapies Inhibiting the EGFR and RAS/RAF Signaling Pathway

From the very early days of our understanding of the role of *BRAF* mutation in mCRC, the main issue associated with mutant-type *BRAF* mCRC has been its dismal prognosis [80]. *BRAF* V600E mutation is associated with an older age, a female gender, right-sidedness, and a Caucasian ethnicity [123–126]. Additionally, *BRAF* V600E mutation leads to diminished DNA mismatch repair via the hypermethylation of the MLH1 gene promoter [127–129]. The *BRAF* V600E mutation is observed in up to 60% of microsatellite instability (MSI)-high tumors and only 5–10% of microsatellite stable (MSS) tumors and is associated with a poor prognosis in both MSS and MSI-high tumors [130]. Regarding *BRAF* non-V600E mutations, the possible association with a poor prognosis has not been clarified [131].

Vemurafenib, an orally bioavailable, adenosine triphosphate (ATP)-competitive, small-molecule inhibitor of BRAF(V600E) kinase, was shown to inhibit cell proliferation and tumor growth in *BRAF* V600E mutant CRC cell lines in vivo and in vitro. Based on this rationale, a phase II trial of vemurafenib monotherapy in patients with *BRAF* V600E mutant mCRC was conducted [132]. However, the efficacy of BRAF inhibition using vemurafenib monotherapy was insufficient, with an RR of 5% and a median PFS of 2.1 months. This lack of efficacy can be explained by the hypothesis that resistance via feedback activation of EGFR may result in the reactivation of the MAPK signaling pathway. Preclinical models have demonstrated a synergistic effect via decreased MAPK signaling with the combined inhibition of BRAF and EGFR [133, 134]. A pilot study of vemurafenib plus panitumumab demonstrated a modest RR (12.5%) [135]. In addition to the rationale behind BRAF and EGFR inhibition, targeting

Table 2 Clinical studies examining anti-EGFR antibody re-challenge in patients with wild-type *KRAS* mCRC

	Phase	Eligibility	EGFR therapy interval	Treatment	*N*	RR (%)	PFS (months)	OS (months)
Santini et al. [115]	II	Wild-type *KRAS* CR, PR, SD > 6 months	Not specified	Cetu + IRI	39	53.8	6.6	NA
JACCRO-CC08 [116]	II	Wild-type *KRAS* CR, PR, SD > 6 months	Not specified	Cetu + IRI	36	2.9	2.4	8.1
JACCRO-CC09 [117]	II	Wild-type *KRAS* CR, PR, SD > 6 months	Not specified	Pani + IRI	25	8.3	3.1	8.9
HGCSG1101 [118]	II	Wild-type *KRAS*	Not specified	Pani	33	6.5	1.9	8.9
CRICKET [119]	II	Wild-type *RAS/BRAF* CR, PR, PFS > 6 months	> 4 months	Cetu + IRI	28	21.5	3.4	9.8
E-Rechallenge [120]	II	Wild-type *RAS* CR, PR, SD > 6 months	> 16 weeks	Cetu + IRI	33	15.2	2.9	8.7

Cetu cetuximab, *CR* complete response, *EGFR* epidermal growth factor receptor, *IRI* irinotecan, *mCRC* metastatic colorectal cancer, *NA* not available, *OS* overall survival, *Pani* panitumumab, *PFS* progression-free survival, *PR* partial response, *RR* response rate, *SD* stable disease

MEK, which is active downstream of BRAF, enabled an improvement in PFS and OS, compared with conventional chemotherapy, in patients with melanoma [136]. In *BRAF* V600E mutant mCRC, a phase I/II trial of dabrafenib combined with trametinib demonstrated a feasible RR (12%) [137]. Furthermore, a phase II trial (SWOG S1406) revealed that the addition of vemurafenib to cetuximab and irinotecan significantly prolonged PFS (HR 0.42; 95% CI 0.26–0.66; $P < 0.001$) in patients with *BRAF* V600E mutant mCRC [138]. Activation of the PI3 K/AKT pathway has been reported to be a mechanism of resistance to BRAF inhibition, and the combined inhibition of BRAF, EGFR, and PIK3A had a synergistic effect in preclinical models [139, 140].

Further synergistic efficacy has been expected for various approaches to MAPK signaling inhibition. Several early phase studies were conducted in patients with *BRAF* V600E mutant mCRC, and promising results were reported (Table 3) [140–142]. Although the addition of alpelisib to encorafenib and cetuximab treatment provided a PFS benefit, it caused additional toxicity and unfortunately did not improve survival (HR 1.21; 95% CI 0.61–2.39) [141]. An ongoing phase III trial (BEACON, NCT02928224) to evaluate encorafenib and cetuximab plus or minus binimetinib versus chemotherapy plus cetuximab in *BRAF* V600E mutant mCRC patients whose disease had progressed after one or two prior regimens should determine the value of triple inhibition including BRAF and EGFR. This study contains a safety lead-in phase in which the safety and tolerability of the combination of encorafenib, binimetinib, and cetuximab will be assessed prior to the phase III part. The clinical outcomes of the patients enrolled in the safety lead-in phase have been reported, with meaningful clinical activity having been observed. Of the 29 patients with *BRAF* V600E mutant mCRC, the confirmed objective RR was 41% and the median period of study treatment was 5.3 months [143].

Regarding *BRAF* non-V600E mutations, *BRAF* non-V600E mutant cancer cells are reliant on tyrosine kinase receptors for their MAPK activation, and the inhibition of EGFR, BRAF and MEK also inhibits cell proliferation and tumor growth in vivo and in vitro [144]. A phase II study of triple combination chemotherapy with encorafenib, binimetinib, and cetuximab in patients with *BRAF* non-V600E mutant mCRC (BIGBANG, UMIN000031857) should provide further useful indications.

5 Conclusions

Anti-EGFR antibodies are key drugs in first-line and later-line treatments for patients with wild-type *RAS* and left-sided mCRC. In second-line treatment, anti-EGFR therapies have been shown to increase the response, but do not confer a survival benefit. Recent efforts to develop non-invasive monitoring techniques, including liquid biopsies, have revealed the potency of monitoring response or resistance to anti-EGFR antibodies and re-challenge treatment with anti-EGFR antibodies. Re-challenge therapy with anti-EGFR antibodies using liquid biopsy test may become a standard of care in mCRC patients who responded to anti-EGFR therapy in the first-line. Furthermore, *BRAF*-mutant mCRC is not only a potential subgroup exhibiting resistance to anti-EGFR antibodies, but also a potential subgroup that could benefit from combinations of BRAF, MEK, and other pathway inhibitors. Nowadays, immunotherapy has led to clinical benefits in MSI-high CRC. However, only approximately 5% of mCRC display MSI-high tumors, and immunotherapy is still under development in the vast majority of mCRC. Anti-EGFR therapies may become candidates of combination drugs for immunotherapy in the future due to their immunomodulatory functions [145–147]. The EGFR and RAS/RAF pathway is a pivotal pathway in mCRC pathogenesis and treatment, and further investigations not only focusing on the direct

Table 3 Clinical studies examining triple combination therapy targeting the EGFR and RAS/RAF pathway in patients with mutant *BRAF* V600E mCRC

	Phase	Treatment	*N*	RR (%)	mPFS (months)
van Geel et al. [140]	Ib	Cetuximab + encorafenib + alpelisib	28	17.9	4.2
		Cetuximab + encorafenib	26	19.2	3.7
Tabernero et al. [141]	II	Cetuximab + encorafenib + alpelisib	52	26.9	5.4
		Cetuximab + encorafenib	50	22.0	4.2
Atreya et al. [142]	II	Panitumumab + dabrafenib + trametinib	35	25.7	4.1
		Panitumumab + dabrafenib	20	10.0	3.4
		Panitumumab + trametinib	19	0.0	NA

EGFR epidermal growth factor receptor, *mCRC* metastatic colorectal cancer, *mPFS* median progression-free survival, *NA* not available, *RR* response rate

inhibition of this pathway, but also combination therapy with targeting of the tumor microenvironment are needed to improve the insufficient prognosis in mCRC patients.

Compliance with Ethical Standards

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