Overcoming the Hurdles: Surmounting Acquired Resistance to Anti-EGFR Therapy in Metastatic Colorectal Cancer

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Abstract: Colorectal cancer is the third most prevalent cancer type in the United States, with an alarming incidence and mortality rate, especially among individuals younger than 50 years. The epidermal growth factor receptor (EGFR), essential for cell proliferation and survival, has surfaced as a promising therapeutic target for metastatic colorectal cancer and has demonstrated success in various clinical trials. Monoclonal antibodies such as cetuximab and panitumumab have proven to be effective against EGFR by blocking vital downstream signaling pathways and inhibiting gene transcription and cell proliferation. Despite this promise, most patients eventually develop resistance to anti–EGFR treatment, thereby limiting its long-term efficacy. Genomic alterations, such as mutations in *KRAS*, *NRAS*, and *BRAF*, often bypass the EGFR receptor, promoting resistance to therapy. Although our understanding of primary resistance to anti-EGFR therapy has improved, acquired resistance remains a significant hurdle. This review explores the potential mechanisms underpinning this acquired resistance and strategies to overcome it.

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in both men and women within the United States.[1] In 2023, an estimated 153,020 new cases of CRC and 52,550 related deaths are projected. The incidence of CRC increases with age, with the highest rates occurring in individuals aged 50 years and older. However, alarming statistics predict that 19,550 diagnoses and 3750 deaths from CRC will occur in individuals younger than 50 years, making it the leading cause of cancer-related death among young adults.

The epidermal growth factor receptor (EGFR), a receptor tyrosine

kinase of the ERBB protein family, is crucial in promoting cell proliferation, migration, angiogenesis, adhesion, and survival.[2,3] Because these pathways are crucial for the growth and survival of cancer cells, EGFR has emerged as a promising therapeutic target for metastatic CRC (mCRC), as seen in multiple clinical trials.[4-6]

Monoclonal antibodies (mAbs), such as cetuximab (Erbitux, Lilly) and panitumumab (Vectibix, Amgen), selectively target EGFR by competing with natural ligands, such as the epidermal growth factor.[7] Cetuximab is a chimeric (mouse/human) immunoglobulin G1 (IgG1) mAb, and panitumumab is a fully humanized IgG1 mAb. They work by inhibiting the ligand-binding activated phosphorylation of EGFR. This inhibition blocks downstream signaling pathways, including the phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway and the RAS/RAF/mitogen-activated protein kinase (MAPK) pathway, resulting in the inhibition of gene transcription and cell proliferation.

Although EGFR is an established therapeutic target in mCRC, most patients who benefit from anti-EGFR treatment will eventually develop resistance (Figure 1). It has become standard practice to test for mutations in genes such as *KRAS*, *NRAS*, and *BRAF* before initiating anti-EGFR treatment.[8,9] These mutations can activate downstream signaling pathways that bypass the EGFR receptor, making tumor cells resistant to anti-EGFR therapy.[10] Additionally, the location of the CRC primary tumor significantly affects anti-EGFR therapy response, with left-sided cancers responding more favorably.[11] Differences in gene expression and molecular subtypes may explain the poorer response in right-sided mCRC.[12] Higher *AREG* and *EREG* gene expression, which is linked to improved response, is more common in left-sided mCRC.[13,14] Thus, the reliance on EGFR-dependent signaling in left-sided mCRC and a less sensitive mutational profile in right-sided mCRC contribute to this variable therapeutic response. However, this assertion is not universally applicable. Data from the biomarker study of the phase 3 PARADIGM trial indicate that patients with right-sided tumors may indeed respond to anti-EGFR therapies, provided they lack gene alterations associated with resistance to anti-EGFR treatments.[15]

Because our understanding of primary resistance mechanisms to anti-EGFR therapy has significantly improved, we are better equipped to select patients most likely to benefit from this treatment. Nonetheless, acquired resistance remains a challenge, limiting the long-term benefits of this therapy. This review examines the potential mechanisms underlying acquired resistance to anti-EGFR therapy and explores strategies to overcome this resistance.

Mechanisms of Acquired Resistance

KRAS *Mutations*

Secondary *KRAS* gene mutations are the most common mechanism of acquired resistance to anti-EGFR therapy, accounting for more than 50% of cases.[16] Many studies have demonstrated that patients with *KRAS*/*BRAF* wild-type (WT) mCRC treated with anti-EGFR therapy develop novel *KRAS* mutations. These can be detected on repeat biopsy at the time of disease progression.[17,18] Importantly, the rate of resistance to anti-EGFR therapy varies based on whether it is given alone or in combination with chemotherapy. A recent review of 3 large randomized clinical trials of patients with *KRAS*/*BRAF*-WT mCRC showed that acquired mutations occurred far more frequently with anti-EGFR monotherapy (46%) than with anti-EGFR therapy in combination with chemotherapy (9%).[19] Mutations were most commonly observed in the *KRAS* gene, followed by the *EGFR* and *BRAF* genes. Of note, acquired *NRAS* gene mutations were rarely seen in mCRC patients on anti-EGFR monotherapy and were not observed in patients on anti-EGFR therapy and chemotherapy.[19] Parseghian and colleagues demonstrated that contrary to popular belief, acquired anti-EGFR therapy resistance did not arise from the growth of resistant subclones but through epithelial-to-mesenchymal transition, which confers resistance to chemotherapy.[19] The elucidation of this molecular resistance mechanism may prove critical in future approaches to anti-EGFR therapy rechallenge.

EGFR *Mutations*

Secondary *EGFR* gene mutations have also been reported to cause acquired resistance to anti-EGFR therapy in mCRC. The development of resistance to EGFR blockade can be attributed to the appearance of *KRAS*/*NRAS* mutations or the formation of EGFR extracellular domain variants that interfere with antibody binding.[10] Arena and colleagues described the emergence of novel *EGFR* ectodomain mutations (including S492R, R451C, and K467T) following cetuximab treatment in 5 patients with mCRC who were *EGFR*-WT at baseline.[18] The *EGFR* S492R and K467T mutations occur in the receptor region and confer resistance to cetuximab by decreasing its binding affinity for EGFR.[18] Although the *EGFR* R451C mutation occurs outside the cetuximab binding site, computational analyses predict that this mutation may disrupt cetuximab binding through the formation of novel disulfide bonds leading to alterations in EGFR tertiary structure.[18] Overall, *EGFR* ectodomain mutations are rare in CRC, occurring in approximately 1% of patients.[20] However, new data suggest that the prevalence of *EGFR* ectodomain mutations in mCRC varies based on whether patients are previously exposed to cetuximab or panitumumab. An analysis of 999 patients in the

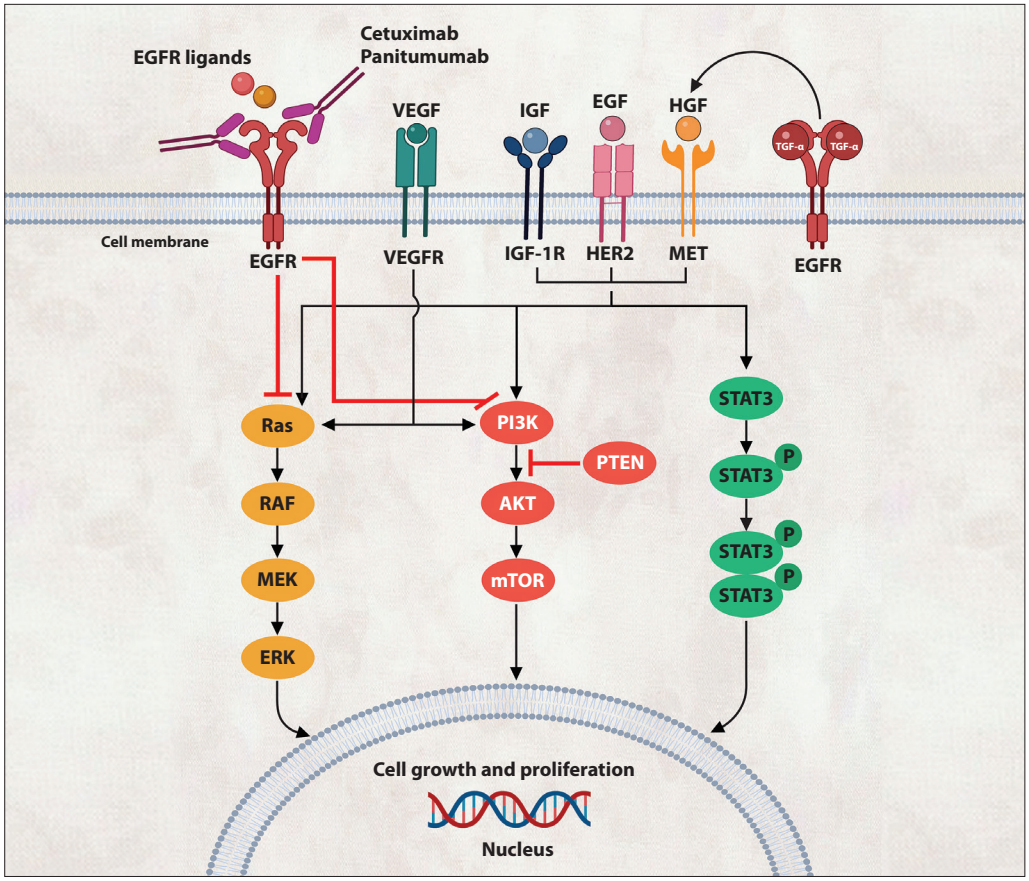

Figure 1. Mechanisms of resistance to anti-EGFR mAbs in mCRC.

EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; mAbs, monoclonal antibodies; mCRC, metastatic colorectal cancer; mTOR, mammalian target of rapamycin; P, phosphorylated; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; STAT3, signal transducer and activator of transcription 3; TGF-α, transforming growth factor alpha; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

ASPECCT study showed that the *EGFR* S492R mutation was preferentially induced in cetuximab-treated patients (16%) vs panitumumab-treated patients (1%).[21] Recently, Parseghian and colleagues demonstrated that acquired *EGFR* gene mutations are common among mCRC patients treated with anti-EGFR therapy alone or in combination with chemotherapy.[19] Interestingly, the S492R mutation does not affect the binding of panitumumab to EGFR, and patients with mCRC who develop *EGFR* S492R mutations can still respond to panitumumab.[22] Most *EGFR* mutations are now identified through next-generation sequencing (NGS) of tumor tissue or circulating tumor DNA (ctDNA) using patient serum samples.[18,19,21]

BRAF *Mutations*

Secondary *BRAF* gene mutations are another important cause of acquired resistance to anti-EGFR therapy. BRAF is a serine/threonine kinase located downstream of the EGFR receptor that contributes to CRC carcinogenesis through activation of the MAPK signaling pathway, resulting in cellular proliferation and enhanced cell survival.[23,24] *BRAF* gene mutations are identified in approximately 10% of patients with mCRC, with more than 95% harboring the V600E activating mutation.[25] Of note, *BRAF* gene mutations are generally mutually exclusive with *RAS* gene mutations.[26] In clinical practice, *BRAF* gene mutations are identified through various methods, including NGS, tissue immunohistochemistry (IHC), and ctDNA.[17,19] Studies suggest that *BRAF* mutations are commonly detected using ctDNA or tissue IHC, and results are generally concordant.[17]

In multiple studies, patients with *BRAF*-WT mCRC who were initially sensitive to cetuximab and irinotecan were found to have developed novel *BRAF* V600E

mutations when biopsied at disease progression.[17,27] Of note, acquired *BRAF* mutations occurred less frequently than acquired *KRAS* mutations.[17] Interestingly, a recent analysis of more than 500 samples from 3 large randomized clinical trials demonstrated that acquired *BRAF* mutations are a more common mechanism of resistance in patients exposed to anti-EGFR monotherapy compared with anti-EGFR therapy in combination with chemotherapy.[19] Additionally, Parseghian and colleagues recently demonstrated the disappearance of *BRAF*-mutated subclones and the development of transcriptomic profiles consistent with epithelial-to-mesenchymal transition.[19,28] This suggests that clonal evolution may not be responsible for acquired resistance to anti-EGFR therapy, and treatment with BRAF inhibitors may be ineffective after receipt of anti-EGFR therapy. Further understanding of resistance mechanisms will be crucial to determining optimal treatment approaches and sequencing of therapies following exposure to anti-EGFR therapy.

HER2 *Amplification*

Human epidermal growth factor receptor 2 (HER2) promotes cellular proliferation by forming EGFR and HER3 heterodimers, leading to the activation of MAPK and AKT/PI3K pathways.[29] HER2 overexpression is present in 3% to 5% of mCRC and represents an uncommon resistance mechanism to anti-EGFR therapy.[29,30] Using patient-derived CRC xenografts, Bertotti and colleagues showed that *HER2* amplification conferred resistance to cetuximab that was reversed by treatment with lapatinib and pertuzumab (Perjeta, Genentech).[31] Others have demonstrated that both *HER2* amplification and increased heregulin ligand secretion serve as resistance mechanisms to anti-EGFR therapy.[32] Given the rarity of HER2 abnormalities in untreated mCRC, *HER2* amplification may occur through clonal evolution, leading to acquired anti-EGFR therapy resistance. Recent studies have confirmed that *HER2* amplification is associated with inferior objective response rate (ORR) and progression-free survival (PFS) in patients with mCRC who are treated with anti-EGFR therapy.[33,34] Clinically, HER2 is typically tested using IHC, in situ hybridization (ISH), or *ERBB2* amplification.[33]

PIK3CA *Mutation*

The *PIK3CA* gene encodes the p110 alpha subunit of PI3K in the AKT/PI3K signaling pathway. *PIK3CA* activating mutations in exon 9 and exon 20 are present in 10% to 20% of patients with CRC. These mutation promote CRC tumorigenesis through constitutive PI3K activation, resulting in uncontrolled cellular proliferation.[35] Owing to conflicting data, the effect of *PIK3CA* mutational status on the response to anti-EGFR therapy is unclear. Although early prospective studies showed no correlation between the presence of the *PIK3CA* mutation and the response to anti-EGFR therapy, larger studies and meta-analyses suggested that *PIK3CA* exon 20 mutations predicted a poor response to anti-EGFR therapy in patients with *KRAS*-WT mCRC.[26,36-38] More recently, acquired *PIK3CA* mutations were observed more frequently in patients with mCRC treated with anti-EGFR therapy who harbored established resistance mutations in *KRAS*, *NRAS*, *BRAF,* or *EGFR* genes.[19] This suggests that *PIK3CA* mutations may be passenger mutations that correlate with tumor mutational burden but do not influence response to anti-EGFR therapy. In clinical practice, *PIK3CA* mutations are most often identified through ctDNA, tissue polymerase chain reaction, or NGS.[19,39]

Loss of PTEN Expression

Phosphatase and tensin homolog (PTEN) is a tumor suppressor protein that serves as an important negative regulator of PI3K signaling. Loss of PTEN expression releases inhibition on PI3K, contributing to CRC development through constitutive PI3K signaling and uncontrolled cellular proliferation.[40] Loss of PTEN expression is observed in 20% to 40% of patients with mCRC, most commonly in microsatellite instability–high (MSI-H) tumors or tumors with high tumor mutational burden.[40-42] Many studies have correlated the loss of PTEN expression with a poor response to cetuximab.[42-44] Interestingly, Loupakis and colleagues found a discrepancy in PTEN expression between the primary CRC and the metastatic site; PTEN loss in the metastasis predicted resistance to cetuximab in *KRAS*-WT patients.[42] Further studies are needed to confirm if PTEN expression in metastases can reliably predict response to anti-EGFR therapy. A more recent study demonstrated that cetuximab-treated mCRC patients with high *AREG* mRNA expression had a shorter time to disease progression if their cancer had a loss of PTEN expression compared with those with intact PTEN expression.[45] This study emphasizes that loss of PTEN expression may be a powerful predictor of anti-EGFR therapy resistance in patients with left-sided mCRC whose tumors strongly rely on the EGFR signaling pathway. Loss of PTEN expression is most often identified through tissue IHC or fluorescence ISH (FISH).[37,45]

IGF-1R Expression

The insulin-like growth factor 1 receptor (IGF-1R) is activated via binding to insulin-like growth factor 1 (IGF-1), resulting in downstream activation of MAPK, PI3K-AKT/mTOR, and STAT3 signaling pathways. IGF-1R also interacts with the EGFR pathway, and *IGF-1R* mutations have been linked with CRC carcinogenesis.[46] Early retrospective studies showed that increased IGF-1 expression

was associated with an inferior ORR to anti-EGFR therapy in patients with *KRAS*-WT mCRC.[47,48] The POSIBA trial subsequently demonstrated that coexpression of MMP-7 and IGF-1R correlated with a poor response to anti-EGFR therapy in patients with mCRC.[49] In addition, the IGF-1 rs2946834 A/G genotype has been linked with failure of anti-EGFR therapy, likely owing to high levels of circulating IGF-1.[50] It is hypothesized that hyperactivation of IGF-1 mediates resistance to anti-EGFR therapy via EGFR-independent activation of PI3K signaling.

MET *Amplification*

The mesenchymal epithelial transition (*MET*) gene is a proto-oncogene that encodes the receptor tyrosine kinase for hepatocyte growth factor and promotes cellular proliferation and metastasis by activating AKT/PI3K and MAPK signaling pathways.[51] Cross talk between the MET and EGFR pathways, as well as *MET* amplification, have been reported as resistance mechanisms to anti-EGFR therapy in mCRC.[51-53] *MET* amplification is common in patients with mCRC who develop resistance to anti-EGFR therapy. Whereas early studies identified *MET* amplification in up to 12.5% of samples from patients with *KRAS*-WT mCRC who were unresponsive to cetuximab, newer studies using ctDNA have demonstrated *MET* amplification in up to 20% of patients with mCRC resistant to anti-EGFR therapy.[51,54] Interestingly, Raghav and colleagues recently showed that *MET* amplification and *KRAS* mutations are rare in patients with mCRC treated with first-line anti-EGFR therapy but are more common in patients receiving these agents in later lines.[55] It is unclear whether *MET* amplification contributes to anti-EGFR therapy resistance via epithelial-to-mesenchymal transition, as previously hypothesized for acquired *KRAS* mutations.[19] Further studies are needed to validate the importance of *MET* amplification in acquired resistance to anti-EGFR therapy.

VEGF Overexpression

Vascular endothelial growth factor (VEGF) promotes the development of CRC through tumor angiogenesis, and its expression is upregulated via EGFR-mediated signaling.[56] VEGF-1 expression is observed in 50% to 70% of patients with CRC, with higher expression seen in advanced disease compared with early-stage disease.[57,58] It has also been implicated in resistance to anti-EGFR therapy in mCRC. Multiple studies showed that increased VEGF receptor 1 (VEGFR-1) expression correlated with resistance to EGFR inhibitors in human CRC cells.[59,60] In addition, silencing of VEGFR-1 restored cetuximab sensitivity in resistant cells, suggesting that VEGFR-1 overexpression was responsible for cetuximab resistance.[60] Early prospective studies of patients with mCRC treated with cetuximab demonstrated that high serum VEGF levels predicted a poor response to treatment.[61,62] Bevacizumab has been shown to inhibit tumor growth by blocking tumor angiogenesis. However, clinical trials of bevacizumab in combination with anti-EGFR therapy failed to show improvements in ORR; they even suggested harm to mCRC patients with shorter PFS and higher rates of grade 3 or 4 toxicity.[63,64] Although VEGF may contribute to anti-EGFR therapy resistance in mCRC, further studies evaluating whether bevacizumab can overcome resistance to anti-EGFR therapies have not been conducted owing to patient safety concerns.

Strategies for Overcoming Resistance to Anti-EGFR Therapy

Strategies to overcome and reverse resistance to anti-EGFR mAbs have been extensively explored in clinical studies (Figure 2). As discussed earlier, compensatory feedback signaling loops produced through alterations in the axes of EGFR downstream signaling pathways and upregulated receptor tyrosine kinases are important mechanisms of resistance to anti-EGFR mAbs.

Targeting* BRAF *Mutations

BRAF serves as a downstream effector of the EGFR/RAS signaling cascade, ultimately leading to the activation of the MAPK/extracellular signal-regulated kinase (ERK) pathway.[65] In CRC, approximately 5% to 9% of cases exhibit *BRAF* mutations, with more than 95% of these mutations occurring in the *BRAF* V600E codon.[66]

Notably, vemurafenib (Zelboraf, Genentech/Daiichi Sankyo), a selective oral inhibitor of BRAF V600E, has demonstrated promising outcomes in patients with metastatic melanoma.[67] However, the response to single-agent BRAF inhibitors or their combination with MEK inhibitors, such as trametinib (Mekinist, Novartis), has been minimal for CRC treatment.[68]

Crucially, the discovery of adaptive feedback following BRAF inhibition, which leads to increased signaling via the EGFR pathway, has been instrumental in the development of studies investigating the combination of BRAF and EGFR inhibitors rather than the administration of BRAF inhibitors alone.[69] Subsequently, numerous trials have been conducted to assess the role of these inhibitors in mCRC. Most notably, the phase 2 randomized SWOG S1406 trial examined the efficacy of irinotecan and cetuximab with or without vemurafenib in patients with *BRAF*-mutant mCRC.[70] The trial reported promising results, with an ORR of 17% vs 4% and a disease control rate (DCR, response or stable disease) of 65% vs 21% in the experimental and control arms, respectively. Furthermore, the primary endpoint of PFS was significantly improved with the addition of vemurafenib, yielding a

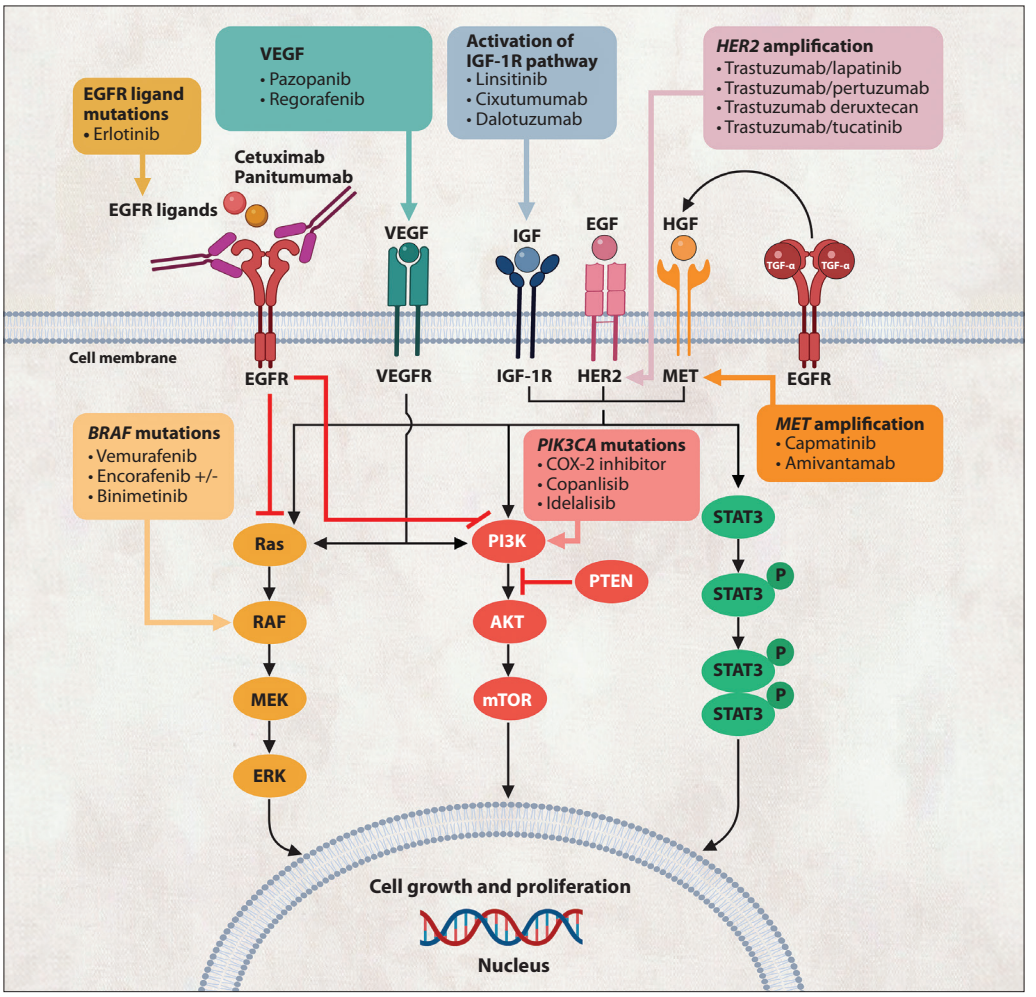

Figure 2. Strategies to overcome resistance to anti-EGFR mAbs in mCRC.

COX-2, cyclooxygenase 2; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IGF-1R, insulin-like growth factor 1 receptor; mAbs, monoclonal antibodies; mCRC, metastatic colorectal cancer; mTOR, mammalian target of rapamycin; P, phosphorylated; PTEN, phosphatase and tensin homolog; PI3K, phosphoinositide 3-kinase; STAT3, signal transducer and activator of transcription 3; TGF-α, transforming growth factor alpha; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

hazard ratio (HR) of 0.50 (95% CI, 0.32-0.76; *P*=.001).

In comparison, encorafenib (Braftovi, Pfizer), another oral BRAF inhibitor, demonstrated a longer half-life and an improved safety profile relative to vemurafenib.[71] The phase 3 randomized BEACON trial assessed the treatment efficacy of various combinations, including the triplet regimen of encorafenib, the MEK inhibitor binimetinib (Mektovi, Pfizer), and cetuximab; the doublet regimen of encorafenib and cetuximab; or the investigators' choice of either cetuximab and irinotecan or cetuximab and leucovorin, 5-fluorouracil, and irinotecan (FOLFIRI), in the second- or third-line metastatic settings.[72] The study revealed an improvement in survival for the triplet group, with a median overall survival (OS) of 9.0 months for the triplet therapy compared with 5.4 months for the control group (*P*<.001). Interestingly, the doublet therapy group exhibited a similar survival outcome, with a median OS of 8.4 months compared with 5.4 months for the control group (*P*<.001). Owing to the findings from the BEACON trial and the superior safety profile observed in the doublet therapy group compared with the triplet therapy group, the US Food and Drug Administration (FDA) approved the combination of cetuximab and encorafenib in the treatment of adult patients with *BRAF* V600E–mutated mCRC.

It is important to note that atypical, non-V600 *BRAF*

(aBRAF) mutations represent a rare molecular subtype of mCRC. Preliminary studies suggest that these mutations exhibit distinct signaling mechanisms that render BRAF inhibition less effective for aBRAF tumors.[73]

In mCRC, understanding and overcoming anti-EGFR resistance is crucial to developing more effective, durable therapies, thereby significantly improving the prognosis for patients diagnosed with cancers that overexpress EGFR. Furthermore, the research and strategies aimed at combating anti-EGFR resistance could also provide valuable insights applicable to resistance mechanisms associated with other targeted therapies.

Targeting RAS *Mutations*

Mutations within *KRAS* and *NRAS* can result in a constitutively active RAS protein that operates independently of upstream signals, leading to the failure of EGFR-targeted therapies.[74] In recent years, significant efforts have been devoted to directly targeting RAS proteins.

One promising candidate is sotorasib (Lumakras, Amgen), a small molecule that inhibits *KRAS* G12C oncogenic signaling by covalently binding to the switch 2 region, which is only present in the inactive GDP-bound conformation. This effectively traps *KRAS* G12C in its inactive state.[75] The CodeBreaK100 trial, a phase 1 study of sotorasib, involved 129 patients with advanced solid tumors harboring the *KRAS* G12C mutation, including 42 patients with mCRC. In this subgroup, 7.1% of patients (n=3) exhibited a confirmed response, 73.8% of patients (n=31) experienced disease control, and the median PFS was 4.0 months (range, 0.0+ to 11.1+).[76] The subsequent phase 2 trial enrolled 62 patients with *KRAS* G12C–mutant mCRC and demonstrated an objective response in 6 (9.7%; 95% CI, 3.6-19.9) of 62 patients, all with partial responses (PRs).[77] Building on the encouraging results from the CodeBreak100 trial, the phase 1b/2 CodeBreak101 trial is currently assessing the safety and tolerability of sotorasib as monotherapy and in combination with other anticancer therapies in patients with *KRAS* G12C–mutant advanced solid tumors.[78] CodeBreak101 features an array of experimental arms, including a sotorasib/trametinib/panitumumab arm, a sotorasib/panitumumab with or without FOLFIRI arm, and a sotorasib/bevacizumab plus FOLFIRI or FOLFOX arm, among others (NCT04185883).

Another notable agent is adagrasib (Krazati, Mirati Therapeutics), also knowns as MRTX849, which covalently and selectively inhibits *KRAS* G12C by binding the mutant protein in its inactive, GDP-bound state.[79] In the KRYSTAL-1 phase 1/2 trial, adagrasib was evaluated as monotherapy and in combination with cetuximab in patients with previously treated mCRC with mutant *KRAS* G12C.[80] The study observed antitumor activity in heavily pretreated patients with *KRAS* G12C–mutated mCRC, both as oral monotherapy and as combination therapy with cetuximab. In the monotherapy group (43 evaluable patients), 19% of patients responded (95% CI, 8-33), with a median response duration of 4.3 months (95% CI, 2.3-8.3) and a median PFS of 5.6 months (95% CI, 4.1-8.3). Conversely, in the combination therapy group (28 evaluable patients), the response rate was 46% (95% CI, 28 to 66), with a median response duration of 7.6 months (95% CI, 5.7 to not estimable) and a median PFS of 6.9 months (95% CI, 5.4-8.1).

Onvansertib, a PLK1-specific ATP competitive inhibitor, has demonstrated the capacity to regulate cell cycle progression, induce mitotic arrest leading to cell death, and modulate tumor growth.[81] A phase 1b/2 study examining the safety and efficacy of onvansertib in combination with FOLFIRI/bevacizumab as a second-line treatment for *KRAS*-mutant mCRC yielded meaningful results.[82] At the recommended phase 2 onvansertib dose of 15 mg/m^2, 31% of patients experienced a PR or complete response (CR), 63% maintained stable disease, and just 6% exhibited progressive disease. Regarding survival, the median PFS across all response-evaluable patients was 9.4 months.[82] PRs also carried over to the different *KRAS*-mutant variants seen in the study subjects, including *KRAS* G12D, *KRAS* G12V, and *KRAS* G13D, which are commonly observed in mCRC (NCT03829410).

Targeting HER2 *Amplification*

In preclinical studies, abnormal activation of HER2 signaling, either through *ERBB2* gene amplification or overexpression of the HER3-activating ligand heregulin, led to continuous activation of the ERK1/2 pathway, which in turn hindered cetuximab-mediated growth inhibition.[83] Nevertheless, a study involving xenograft cohorts from 85 patient-derived mCRC samples showed that *HER2*-amplified tumors were responsive to HER2 blockade.[31] Anti-HER2 monotherapy using pertuzumab or the reversible tyrosine kinase inhibitor (TKI) lapatinib had limited effectiveness against *HER2*-amplified CRC xenografts; however, the combination of lapatinib with either pertuzumab or cetuximab provided a better response.

Initial clinical trials of trastuzumab in mCRC investigated the integration of this mAb with chemotherapy. Clark and colleagues evaluated the combination of FOLFOX and trastuzumab for second- or third-line treatment of HER2-positive mCRC.[77] At the same time, another phase 2 study explored the pairing of trastuzumab and irinotecan for patients with HER2-positive mCRC who had previously received 1 line of therapy.[84,85] Unfortunately, both trials were terminated early, the FOLFOX plus trastuzumab study owing to insufficient efficacy and the irinotecan plus trastuzumab study owing

to low patient enrollment. In light of these disappointing outcomes, subsequent studies focused on the potential of dual HER2 blockade.

The HERACLES series comprised several phase 2 clinical trials that examined various anti-HER2 treatments. HERACLES cohort A assessed the combined effect of trastuzumab and lapatinib on patients with *KRAS* exon 2 WT mCRC who exhibited *HER2* amplification and/or overexpression and were resistant to standard treatment.[86] Among the 32 treated patients, the results revealed an ORR of 28%, a DCR of 69%, a median PFS of 4.7 months, and a median OS of 10 months. Conversely, HERACLES cohort B explored the combination of pertuzumab and the antibody-drug conjugate trastuzumab emtansine, also known as T-DM1 (Kadcyla, Genentech) in 31 *KRAS* and *BRAF*-WT HER2-positive mCRC patients whose disease was refractory to standard therapies.[87] The primary endpoint was not achieved, with an ORR below the anticipated rate of 30% and higher (9.7%). Stable disease was observed in 67.7% of patients, and the DCR was 77.4%. The median PFS of 4.2 months was comparable to the 4.7-month PFS seen in the HERACLES-A cohort.

The MyPathway trial is a phase 2 basket trial including multiple solid tumors. The researchers enrolled 57 patients with *HER2*-amplified mCRC receiving a combination of trastuzumab and pertuzumab.[88] Overall, 18 patients (32%) achieved an objective response; in 4 cases, this response was longer than 12 months. The results obtained in the HERACLES-A and MyPathway trials led to the inclusion of trastuzumab/lapatinib and trastuzumab/pertuzumab regimens in the National Comprehensive Cancer Network guidelines for treating mCRC.[89]

Trastuzumab deruxtecan, also known as T-DXd or DS-8201 (Enhertu, Daiichi-Sankyo/AstraZeneca), is an antibody-drug conjugate combining a humanized anti-HER2 antibody with a topoisomerase I inhibitor. This agent was studied for its antitumor activity and safety in the phase 2 DESTINY-CRC01 trial.[90] The trial included patients with HER2-positive *KRAS*/*BRAF*-WT mCRC whose disease had progressed on 2 or more lines of treatment; some of these patients were pretreated with other anti-HER2 agents. In total, 78 patients were enrolled: 53 in cohort A (HER2 IHC 3+ or 2+ with positive ISH), 7 in cohort B (IHC 2+ with negative ISH), and 18 in cohort C (IHC 1+). After a median follow-up of 27.1 weeks, cohort A had an ORR of 45.3% (95% CI, 31.6-59.6), and patients pretreated with anti-HER2 agents also achieved a high ORR of 43%. No responses were observed in cohorts B and C. With an updated longer-term median follow-up of 62.4 weeks and 86 patients treated, the ORR of cohort A was 45.3%, the DCR was 83%, the median PFS was 6.9 months, and median OS was 15.5 months.

Recently, the MOUNTAINEER trial examined the combination of trastuzumab and tucatinib (Tukysa, Seagen),[91] an oral TKI targeting the HER2 protein, in advanced HER2-positive mCRC patients. In the treatment arm consisting of 84 patients who received trastuzumab in combination with tucatinib, the confirmed ORR was 38.1% (95% CI, 27.7-49.3), as assessed by blinded independent central review. The median duration of response was 12.4 months (interquartile range, 8.3-25.5), the median PFS was 8.2 months (95% CI, 4.2-10.3), and the median OS was 24.1 months (95% CI, 20.3-36.7).[28] These results led to FDA-accelerated approval for patients with HER2-positive mCRC whose disease progressed on standard therapy.

PI3K Activation, Loss of PTEN Expression, and PI3K Inhibitors

Mutations in the PI3K pathway, particularly the aberrant activation of AKT/mTOR and loss of PTEN expression, have been implicated in the development of resistance to anti-EGFR mAbs in mCRC.[92] The *PIK3CA* gene, which encodes the PI3K enzyme, frequently exhibits mutations in exons 9 and 20.[26] Interestingly, exon 20 mutations have been associated with a worse prognosis in patients with *KRAS*-WT mCRC treated with cetuximab. In contrast, exon 9 mutations do not appear to impact survival outcomes.[93]

Although initial clinical trials evaluating the combination of PX-866 (a pan isoform PI3K inhibitor) and cetuximab yielded disappointing results in terms of PFS and OS for patients with *KRAS*-WT mCRC, recent studies have shown promising developments.[94] These early trials also indicated increased toxicity, highlighting the need for alternative therapeutic approaches.

One such approach is the ongoing phase 1/2 trial investigating copanlisib (Aliqopa, Bayer), a highly selective pan-class I PI3K inhibitor, in combination with the anti–programmed death 1 antibody nivolumab (Opdivo, Bristol Myers Squibb).[95] This study focuses on treating relapsed/refractory solid tumors, including microsatellite-stable CRC (NCT03711058). Simultaneously, the active phase 1b/2 C-PRECISE-01 study is evaluating MEN1611, another PI3K inhibitor, in combination with cetuximab for patients with *PIK3CA*-mutated, *RAS*- and *RAF*-WT mCRC, whose disease has previously failed to respond to irinotecan, oxaliplatin, 5-fluorouracil, and anti-EGFR–containing regimens (NCT04495621). Another ongoing phase 2 study is evaluating the efficacy of GSK2636771, a class I PI3K beta inhibitor, in cancers with loss of PTEN expression, including advanced and refractory solid neoplasms (NCT04439188).

In addition to these targeted therapies, experimental data suggest that aspirin may play a role in modulating the PI3K pathway.[96] Aspirin has been found to suppress

prostaglandin-endoperoxide synthase 2 and downregulate PI3K signaling activity.[97] Notably, low-dose aspirin significantly improved survival outcomes in patients with *PIK3CA*-mutated CRC, whereas no such effect was seen in patients with *PIK3CA*-WT CRC.[98] This finding highlights the potential of repurposing existing drugs to enhance the efficacy of targeted therapies in mCRC.

MET *Amplification/Activation and MET Inhibitors*

MET mutations and amplification are rarely discovered in patients with CRC, with rates of 2% to 5% and 0.5% to 2%, respectively.[20,99] For this reason, *MET* amplification cannot be considered a reliable biomarker of primary resistance to anti-EGFR therapy in mCRC. Acquired resistance to anti-EGFR therapies in CRC may arise owing to the emergence of *MET* amplification, which could result from the expansion of preexisting *MET*-amplified clones under anti-EGFR treatment pressure.[100] This resistance, as demonstrated by negative responses to cetuximab in both patient samples and xenografts, highlights the therapeutic potential of combining MET inhibitors with anti-EGFR agents.[51,101]

Tivantinib (ARQ 197), a selective non-ATP competitive c-MET inhibitor, was studied in combination with cetuximab in a phase 2 trial (NCT01892527) enrolling patients with *MET*-amplified, previously treated *KRAS*-WT mCRC.[102] Although the trial's first stage showed a promising DCR of 52.4%, the primary endpoint was not reached during the second stage. Only 4 patients achieved an objective response. However, survival results were encouraging, with a median PFS of 2.6 months and a median OS of 9.2 months.

In contrast, rilotumumab, a humanized IgG mAb targeting hepatocyte growth factor, was investigated in a randomized phase 1b/2 trial comparing rilotumumab or the anti-IGF-1R antibody ganitumab with panitumumab in patients with *KRAS*-WT mCRC.[103] The combination of rilotumumab and panitumumab did not yield significant benefits in median OS (13.8 vs 13.7 months; *P*=.71) for patients with MET-high disease compared with those with MET-low disease.

Cabozantinib (Cabometyx, Exelixis), an oral multi-TKI targeting several tyrosine kinases, including MET, RET, and VEGFR-2, demonstrated significant antitumor activity in xenograft and cell line models of CRC.[104] The phase 1b multitumor cohort study COSMIC-021 (NCT03170960) evaluated cabozantinib plus the anti–programmed death ligand 1 mAb atezolizumab (Tecentriq, Genentech).[105] In the CRC cohort (n=31), the results showed an ORR of 10%, a DCR of 71%, a median PFS of 3.0 months, and a median OS of 14.0 months. Notably, patients with *RAS*-WT (n=12) exhibited longer PFS and OS than those with *RAS* mutations (n=19).

Amivantamab (Rybrevant, Janssen), a fully human bispecific antibody targeting both EGFR and MET, has demonstrated clinical effectiveness against tumors exhibiting primary activating *EGFR* mutations, *EGFR* resistance mutations, or MET pathway activation.[106,107] Encouraging outcomes have been observed in clinical trials of *EGFR*-mutant non–small cell lung cancer when amivantamab is combined with lazertinib, a third-generation, brain-penetrating EGFR TKI, as evidenced by the CHRYSALIS study (NCT02609776) and the phase 3 MARIPOSA trial (NCT04487080), which led to FDA approval.[108] The ongoing OrigAMI-1 trial (NCT05379595), a phase 1b/2 study, aims to evaluate the safety, tolerability, and antitumor efficacy of amivantamab, both as a stand-alone therapy and in conjunction with standard chemotherapy, for patients with advanced CRC or mCRC harboring WT *KRAS*, *NRAS*, *BRAF*, and *EGFR*.[109]

ctDNA Monitoring and Anti-EGFR Rechallenge

The addition of anti-EGFR therapy has led to significantly improved survival outcomes in patients with *KRAS*/*NRAS*-WT mCRC. Nevertheless, acquired genetic aberrations eventually emerge, resulting in secondary resistance.[28] Owing to recent advancements in ctDNA testing, the noninvasive detection of various molecular alterations has become feasible, elucidating the mechanisms underlying the development of resistance to targeted therapies in mCRC.[110]

In the phase 2 PROSPECT-C study, which assessed the use of single-agent cetuximab in *RAS*-WT mCRC, almost 50% of the 22 patients eligible for analysis displayed RAS pathway aberrations in their baseline cell-free DNA (cfDNA), including *KRAS*/*NRAS*, *BRAF* V600E, *PIK3CA* E545K mutations, and *ERBB2* amplification.[111] The presence of RAS pathway aberrations in baseline cfDNA was significantly correlated with reduced PFS and OS. Furthermore, emerging subclonal RAS pathway aberrations contributed to acquired cetuximab resistance during longitudinal monitoring, including c-MET amplification, *KRAS* Q61H A-T, *KRAS* Q61H A-T, *KRAS* G12D, and polyclonal *RAS* mutations.

In the CO.26 study, 169 patients with treatment-resistant mCRC underwent pre–anti-EGFR tissue whole-exome sequencing and baseline and week 8 ctDNA assessments.[112] Acquired alterations in patients with prior anti-EGFR treatment were compared with those who had not. The study found that 21% of patients with previous anti-EGFR therapy exhibited at least 10 putative concurrent resistance mechanisms, compared with only 5% of patients without prior therapy (*P*=.010). Besides the expected resistance mutations, additional mutations in genes such as *ZNF217*, *MAP2K1*, *PIK3CG*, *LRP1B*, *ATM*, *ATR*, and *BRCA1* were observed.

In a retrospective analysis carried out at the MD Anderson Cancer Center, 135 patients with *RAS*/*EGFR*/*BRAF*-WT mCRC underwent anti-EGFR treatment and later exhibited progression.[28] Plasma samples were collected for ctDNA sequencing, and the results demonstrated that the relative mutant allele frequency for *RAS* and *EGFR* decreased exponentially, displaying a cumulative half-life of 4.4 months. The results highlight the potential strategy of anti-EGFR rechallenge.

The multicenter, single-arm phase 2 CRICKET trial enrolled 28 patients with *RAS*- and *BRAF*-WT mCRC, incorporating preplanned ctDNA analysis, and administered third-line cetuximab plus irinotecan treatment after acquiring resistance to the same regimen in the first-line setting.[113] The study demonstrated that the absence of *RAS* mutations in ctDNA before cetuximab rechallenge was associated with a 31% response rate. Furthermore, ctDNA analysis indicated that none of the patients who still had detectable *RAS* mutations at the time of rechallenge responded to the treatment.

Recently, the single-arm phase 2 CHRONOS clinical trial was designed to evaluate whether detecting ctDNA *RAS*/*BRAF*/*EGFR* mutations could inform decisions regarding anti-EGFR rechallenge in patients with WT mCRC.[114] Among the 27 enrolled patients, 8 (30%) achieved a PR and 40% of patients experienced stable disease, which persisted for more than 4 months in 82% of these patients. Furthermore, the most prevalent resistance mechanisms seen were mutations or amplifications of *EGFR*, *KRAS*, and *NRAS*, which affected 48% of patients, along with *PTEN* mutations and *MET* amplification. Overall, the CHRONOS study highlighted the advantage of using ctDNA to guide patient selection for anti-EGFR rechallenge and monitor their subsequent tumor response.

The role of ctDNA is continually evolving, and enhancing our understanding of acquired resistance may potentially lead to improved mCRC outcomes. By employing comprehensive ctDNA panels that can monitor tumor kinetics and detect resistance before radiographic progression occurs, it is possible to refine patient selection for anti-EGFR rechallenge. Patient selection can be refined by identifying both *RAS*/*BRAF*/*EGFR* alterations and non-*RAS*/*BRAF*/*EGFR* alterations that could negate potential benefit.

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