Anti-EGFR therapy in metastatic colorectal cancer: mechanisms and potential regimens of drug resistance

Qing-Hai Li1, Ying-Zhao Wang1, Jian Tu2, Chu-Wei Liu1, Yu-Jie Yuan1, Run Lin3, Wei-Ling He1, Shi-Rong Cai1, Yu-Long He1 and Jin-Ning Ye1,*

1Department of Gastrointestinal Surgery, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P. R. China; 2Department of Musculoskeletal Oncology, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P. R. China; 3Department of Radiology, the First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, P. R. China

*Corresponding author. Department of Gastrointestinal Surgery, The First Affiliated Hospital of Sun Yat-sen University, 58 Zhongshan Road, Guangzhou, Guangdong 510080, P. R. China. Tel: +86-13632480199; Email: yejn@mail.sysu.edu.cn

Abstract

Cetuximab and panitumumab, as the highly effective antibodies targeting epidermal growth factor receptor (EGFR), have clinical activity in the patients with metastatic colorectal cancer (mCRC). These agents have good curative efficacy, but drug resistance also exists at the same time. The effects of KRAS, NRAS, and BRAF mutations and HER2 amplification on the treatment of refractory mCRC have been elucidated and the corresponding countermeasures have been put forward. However, the changes in EGFR and its ligands, the mutations or amplifications of PIK3CA, PTEN, TP53, MET, HER3, IRS2, FGFR1, and MAP2K1, the overexpression of insulin growth factor-1, the low expression of Bcl-2-interacting mediator of cell death, mismatch repair-deficient, and epigenetic instability may also lead to drug resistance in mCRC. Although the emergence of drug resistance has genetic or epigenetic heterogeneity, most of these molecular changes relating to it are focused on the key signaling pathways, such as the RAS/RAF/mitogen-activated protein kinase or phosphatidylinositol 3-kinase/Akt/mammalian target of the rapamycin pathway. Accordingly, numerous efforts to target these signaling pathways and develop the novel therapeutic regimens have been carried out. Herein, we have reviewed the underlying mechanisms of the resistance to anti-EGFR therapy and the possible implications in clinical practice.

Key words: metastatic colorectal cancer; EGFR; drug resistance; cetuximab; panitumumab

Introduction

Colorectal cancer (CRC) is one of the most common tumors. Data from 185 countries have shown that ~1.8 million new CRC cases and 881,000 deaths were estimated to have occurred in 2018 and the incidence and mortality rank third and second, respectively [1]. In addition, ~20% of new cases are diagnosed as metastatic colorectal cancer (mCRC) at the time of initial visiting [2]. Fortunately, although the incidence and mortality of CRC in some underdeveloped areas are still increasing, they are declining in the whole world, especially in the developed countries [3]. At present, a limited number of targeted agents such as cetuximab, panitumumab, bevacizumab, aflibercept, and regorafenib have been shown to be active in the treatment of the patients with mCRC (hereinafter referred to as the patients or mCRCs). However, a number of the patients benefit a lot from the clinical application of these drugs [4–8].

Cetuximab is a chimeric (mouse/human) IgG1 monoclonal antibody (mAb). As a targeted drug, cetuximab can competitively bind to epidermal growth factor receptor (EGFR) with the...
natural ligands such as EGF [9]. Its main pharmacological mechanism is to inhibit the phosphorylation of EGFR tyrosine kinase caused by the ligands binding and then block a series of reactions such as gene transcription and cell proliferation induced by the activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway and the RAS/RAF/mitogen-activated protein kinase (MAPK) pathway. Cetuximab can also mediate antibody-dependent cellular cytotoxicity and receptor internalization, so as to fully play the antitumor role [10–12]. The major side effects of cetuximab are skin toxicity and venous thromboembolic; skin rash may be related to the prognosis [13–17]. Panitumumab is a recombinant humanized IgG2kappa mAb; both its pharmacological mechanisms and side effects are similar to those of cetuximab. In September 2006, panitumumab was approved by the US Food and Drug Administration as a single agent for the treatment of advanced CRC, but now it is mainly used in the combined-therapy regimens with other chemotherapeutic agents.

Although the use of cetuximab and panitumumab has greatly improved the overall survival (OS) and other clinical outcomes of mCRCs [4, 5], some patients cannot benefit from it. The discovery of the relationship between KRAS gene mutations and resistance to anti-EGFR mAbs has aroused the interest of a large number of scholars in the study of drug resistance. In this review, the molecular mechanisms of the resistance to anti-EGFR mAbs and the potential therapy regimens will be systematically elaborated on from three aspects: abnormal molecules in the EGFR pathway, abnormal activations between the paralleled pathways, and the other mechanisms.

Abnormal molecules in the EGFR pathway

Abnormalities of EGFR and its ligands

EGFR is one of the members of the ERBB/HER protein family that is composed of EGFR (HER1/ERBB1), HER2 (ERBB2/neu), HER3 (ERBB3), and HER4 (ERBB4). It consists of extracellular domains, transmembrane domains, and signal-transduction domains with tyrosine kinase activity. After binding to its ligands through the extracellular binding region, the proteins in the signal-transduction region phosphorylate and finally leads to the intracellular cascade reactions that mainly promote cell proliferation [10, 18, 19]. In principle, the mutations of EGFR, the low expression of EGFR, and the changes in its ligands are detrimental to the efficacy of anti-EGFR therapy in some cancers. For example, in lung cancer, the EGFR gene copy number (GCN) or mutations (especially EGFR T790M) has been confirmed to be related to the resistance of molecule-targeted drugs [20, 21]. In the latest molecular-detection guideline of lung cancer, the importance of both EGFR GCN and mutations detection before the targeted treatment is reiterated [22]. Although some studies have pointed out that patients are all responsive to anti-EGFR mAbs regardless of the status of the EGFR [23–25], emerging evidence has shown that EGFR GCN and the acquired mutations of extracellular domains are related to the resistance to anti-EGFR mAbs in mCRCs [26–31], but EGFR-related detection in mCRCs has not been recommended at present [32]. Therefore, whether this indicator can be used as an efficacy predictor of anti-EGFR therapy in patients should be further investigated.

The ligands of EGFR mainly include EGF, transforming growth factor α (TGF-α), amphiregulin (AREG), epipluron (EREG), epigen (ECPG), β-cellulin, and heparin-binding EGF (HB-EGF). As early as 2007, researchers had noticed that the expression levels of EREG and AREG are related to the efficacy of cetuximab in mCRCs [33]. Subsequently, accumulating studies have confirmed this finding; they have found that the higher the expression levels of these ligands, the better the observed efficacy of anti-EGFR mAbs in mCRCs can be, but this correlation is not consistent in patients with KRAS mutations [34]. In 2016, a randomized clinical trial of panitumumab plus irinotecan and ciclosporin in the treatment of advanced CRC has come out with a new ligand-expression model and pointed out that the expression levels of EREG and AREG are related to the therapeutic efficacy of panitumumab [35]. All of the above studies have suggested that the downregulation of EGFR ligands, especially EREG and AREG, may be one of the mechanisms of resistance to anti-EGFR mAbs. Therefore, the expression levels of EREG and AREG may be valuable markers for the choice of regimens in mCRCs, and it is worthy to be further optimized and then used for clinical guidance in order to reduce drug resistance and improve anti-EGFR mAbs efficacy (Figure 1).

Mutations of RAS

KRAS, HRAS, and NRAS are three major members of the RAS family. RAS as a proto-oncogene encodes the protein with GTPase activity, which plays a role of transducing and self-inactivating the signals in EGF signaling. At present, the abnormalities of the RAS family have been reported to be related to a variety of tumors, and the mutations of KRAS and NRAS closely relate to CRC among them, but the relationship between CRC and the abnormality of HRAS is rarely reported [36]. According to the statistics, ~37%–45% of CRCs harbor KRAS mutations in exon 2 (codons 12 or 13) and nearly 10% of CRCs harbor non-KRAS exon 2 RAS mutations that consist of non-exon 2 KRAS mutations (codons 59 or 61 mutations in exon 3, codons 147 or 117 mutations in exon 4) and NRAS mutations (codons 12 or 13 in exon 2 and codons 59 or 61 in exon 3) [5, 37–42].

Although studies have shown that the status of KRAS and NRAS is independent of the tumor staging [37, 43], since Lievre et al. [44] first reported that the mutations of KRAS can reduce the efficacy of anti-EGFR mAbs in 2006, emerging studies have confirmed that KRAS mutations are the major causes of resistance to anti-EGFR therapy [5, 43, 45–50]. These pieces of evidence are mainly as follows: not only the resistance to anti-EGFR mAbs is related to KRAS mutations in exon 2 codons 12, but also the other mutant types of RAS such as codons 13 mutation in exon 2 and non-KRAS exon 2 RAS mutations can render the resistance to cetuximab or panitumumab in mCRCs; in addition to primary RAS mutations, there are many patients who harbor acquired RAS mutations in response to EGFR blockade. At present, the genotypes of KRAS and NRAS have become the main basis for choosing the chemotherapy regimen in mCRCs; anti-EGFR therapy in patients with KRAS and NRAS wild type (WT) has greatly improved their benefits. At the same time, in order to solve the problem of genes-detection sensitivity and specificity, not only should genes testing be performed only in the laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA-88), but also the latest National Comprehensive Cancer Network (NCCN) Colon/Rectal Cancer Panel and tumor-molecular-detection guidelines have recommended the next-generation sequencing (NGS) technology (Table 1) [32, 51].

The structures of KRAS- and NRAS-mutant proteins are complex; although they have been known for a long time, the research and development of the related agents such as molecule-targeted drugs, mAbs, or vaccines are still quite difficult, and there is no RAS-targeted drug that has been successfully marketed so far. Therefore, the chemotherapy regimens with bevaciuzumab are still recommended in the guidelines for patients harboring RAS mutations. However, there have been several
agents that have deserved our attention in recent years. In 2013, a paper showed that KRAS G12C could be targeted by a covalent compound that locks the mutant protein in its inactive GDP-bound state, which is supported by the discovery that KRAS G12C retains the highest residual intrinsic GTPase activity [52]. In addition, after the development and verification of the tool covalent compounds ARS-853 and ARS-1620, the first specific irreversible covalent inhibitors of KRAS-G12C in the clinic came from Amgen, AMG510, then from Mirati Therapeutics, MRTX849 (Figure 1). These two agents have been studying in clinic trials or in vitro and have been showing excellent antitumor efficacy that includes driving antitumor immunity [53–55]. Some clinic trials still aim to further confirm this evidence (NCT03600883 and NCT03785249). BI-1701963 is a non-specific inhibitor of KRAS; it blocks RAS signal transduction through inhibiting SOS1 selectively. The preclinical study has proved that BI-1701963 is effective for KRAS-mutant tumors. At present, China and other countries are participating in the global early clinical trial of this drug (NCT04111458). Anti-EGFR antibodies and metastatic colorectal cancer

Figure 1. EGFR signaling and potential regimens in cetuximab- or panitumumab-resistant mCRC. In addition to NRAS/KRAS/BRAF mutations, low expression of EGFR/AREG/EREG, the mutations of extracellular domains of EGFR, PIK3CA, PTEN, TP53, and MEK1 are related to cetuximab or panitumumab resistance. The agents targeting BRAF and MEK1/2 have been approved for the subsequent therapy of advanced or metastatic CRC, some other targeted drugs such as PI3K, Mtor, and RAS inhibitors also deserve our attention, especially the KRAS G12C inhibitors AMG-510 and MRTX849. EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; HB-EGF, heparin-binding EGF; TGF-α, transforming growth factor α; AREG, amphiregulin; EREG, epiregulin; ED, extracellular domains; TKD, tyrosine kinase domains; GRB2, growth factor receptor bound-2; SOS, son of sevenless; MEK1/2, mitogen-activated protein kinase kinase; ERK1/2, extracellular-signal-regulated kinase; PI3K, phosphatidylinositol 3-kinase; mTOR, mammalian target of rapamycin; p, phosphorylation; * have been approved for markets.

Mutations of BRAF

BRAF proto-oncogene belongs to the RAF genes family that includes two other members: CRAF and ARAF. As an important component of the RAS/RAF/MAPK pathway, BRAF mediates the combination of RAF and MAPK kinase (MAPKK/MEK1/2) in the signal transduction and regulates cell proliferation [62]. Statistics have shown that 30%–60% of melanoma, 30%–50% of thyroid cancer, and 5%–9% of CRC harbor BRAF mutations. The features of BRAF mutations in CRC are likely to occur in smokers and elderly and female patients whose primary tumor is mainly located in the right colon. Such patients commonly have high-grade cancers at diagnosis and a higher number of cancer-involved lymph nodes; they are more likely to be microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) and to progress rapidly [63].
Table 1. Currently recommended molecular testing for colorectal cancer

| Biomarker | Test defect | Time | Technology | Recommendations | Evidence |
|-----------|-------------|------|------------|----------------|----------|
| KRAS/NRAS<sup>a</sup> | Mutation | Workup for metastatic disease (suspected or proven) | NGS<sup>b</sup>, PCR | Avoid cetuximab or panitumumab treatment in mCRC patients who have tumors with KRAS and NRAS mutations (exons 2, 3, and 4 in both) | NCCN indicates lower-level, uniform acceptance (category 2A); but many believe classification is high-level, uniform acceptance |
| BRAF<sup>b</sup> | Mutation V600E | Workup for metastatic disease (suspected or proven) | NGS, PCR<sup>b</sup> | Cetuximab or panitumumab treatment is not recommended in mCRC patients harboring BRAF<sup>V600E</sup> mutation unless given with BRAF inhibitors with or without MEK inhibitors at the same time | NCCN indicates lower-level, uniform acceptance (category 2A); but many believe classification is high-level, uniform acceptance |
| HER2 | Amplification | Workup for metastatic or advanced disease (suspected or proven) | NGS, IHC, or FISH, need more evidence | Patients with HER2 amplification may be resistance to Cetuximab or panitumumab, trastuzumab plus (pertuzumab or lapatinib) regimen is an option for mCRC patients with HER2 amplified and RAS WT, but need more evidence<sup>d</sup> | NCCN indicates lower-level, wide acceptance (category 2B); but many believe classification is high-level, wide acceptance |
| NTRK | Gene fusion | Workup for metastatic disease (suspected or proven) | NGS, FISH, PCR | Larotrectinib is a treatment option for mCRC patients who are NTRK gene-fusion positive | NCCN indicates lower-level, uniform acceptance (category 2A); but many believe classification is high-level, uniform acceptance |
| MMR/MS | dMMR<sup>c</sup>/MSI-H | All patients with colon or rectal cancer | NGS, IHC<sup>c</sup>, PCR | Stage II MSI-H CRC patients may have a good prognosis but lack of efficacy of fluorouracil-based adjuvant therapy (nivolumab with or without ipilimumab) or pembrolizumab regimens are recommended for the patients with dMMR/MSI-H only | NCCN indicates lower-level, uniform acceptance (category 2A); but many believe classification is high-level, uniform acceptance |

NGS, next-generation sequencing; PCR, polymerase chain reaction; NCCN, National Comprehensive Cancer Network; IHC, immunohistochemistry; MMR, mismatch repair; dMMR, mismatch repair-deficient; MS, microsatellite; MSI-H, microsatellite highly instability; mCRC, metastatic colorectal cancer; FISH, fluorescence in situ hybridization; WT, wild-type.

<sup>a</sup>KRAS and NRAS are determined alongside BRAF mutations.

<sup>b</sup>Testing can be performed on primary and/or metastatic colorectal tissue specimens.

<sup>c</sup>IHC refers to staining tumor tissue for protein expression of the four MMR genes known to be mutated in Lynch syndrome (MLH1, MSH2, MSH6, and PMS2).

<sup>d</sup>If no previous treatment with HER2 inhibitor.

BRAF<sup>V600E</sup> mutations are the most common mutations (about 80%). Among them, BRAF<sup>V600E</sup> accounts for >90% of BRAF<sup>V600E</sup> mutations, which refers to the T-to-A changing at nucleotide 1799 and a substitution of valine to glutamic acid [64]. The result of these changes is the rendering of BRAF protein to be an active monomer that is independent of the upstream signals, causing the synchronous and continuous activation of downstream pathways and promoting cell proliferation and differentiation. Some other rare BRAF-mutant types include mutant-mutant BRAF dimers that lead to high extracellular-signal-regulated kinase (ERK1/2) activation in a RAS-independent manner and mutant BRAF-wild-type CRAF dimers that amplify the signaling downstream of RAS. Many studies have proved that patients harboring BRAF mutations are resistant to cetuximab or panitumumab and have a generally poor prognosis [4, 5, 41, 65–67]. Therefore, BRAF status is a strong predictor for the predictive prognosis of CRC. In addition to RAS typing, high-sensitivity typing of the BRAF gene in mCRCs is equally important (Table 1) [32].

Different from the development of RAS inhibitors, many breakthroughs have been made in the development of BRAF inhibitors. At present, the approved BRAF monomers-targeted inhibitors include vemurafenib, dabrafenib, and encorafenib (LGX818) (Figure 1). They are mainly used in the treatment of melanoma, but the efficacy of single-agent therapy for mCRCs remains unclear [68], which is mainly related to the fact that, after inhibiting the MAPK pathway using BRAF inhibitors, EGFR can be activated by feedback in mCRCs, whereas melanoma only expresses low-level EGFR [69, 70]. However, the emergence of BRAF inhibitors makes the combined-therapy regimens possible. According to the latest NCCN Guidelines of Colon/Rectal Version, for the patients with BRAF<sup>V600E</sup> mutation, the first-line targetted-therapy regimens should include bevacizumab, while the subsequent options include targeting BRAF, MEK, and EGFR at the same time, such as dabrafenib plus trametinib and cetuximab or panitumab, or encorafenib plus binimetinib (MEK162) and cetuximab or panitumumab. These two regimens have been proven to be effective and tolerable, which improves the benefits of the patients to some extent [71–73]; another multi-targeted-therapy regimen [MEK162 plus LGX818 and LEE011 (cyclin-dependent kinase (CDK) 4/CDK6 inhibitor)] is in a phase IB/II clinical trial (NCT01543698). In addition, as
mentioned above, this type of patient situation is often accompanied by dMMR/MSI-H, so that anti-programmed death1 (PD-1)/anti-programmed death ligand 1 (PD-L1) and anti-cytotoxic T-lymphocyte antigen 4 (CTLA-4) immunotherapy may also have an ideal therapeutic effect [63, 74].

**Mutations of PIK3CA, PTEN, and TP53**

The KRAS/NRAS/BRAF status is a strong predictor of anti-EGFR mAbs efficacy in mCRCs, but more predictive factors are needed for the better selection of patients who will benefit highly from anti-EGFR mAbs. PIK3CA encodes p110alpha, a catalytic subunit of PI3K that mediates the PI3K/AKT pathway and promotes cell survival, whereas PTEN and TP53 are negative regulators of this pathway as the tumor-suppressor genes. PTEN plays a role as a phosphatase that dephosphorylates PIP3 or PIP2, and therefore inhibits the activation of PI3K and blocks this pathway [18, 75, 76]. TP53 encodes p53 protein, which acts not only as a guardian of the genome in normal cells, but also as an inhibitor of the oncogenes in cancer cells. Therefore, changes in PIK3CA could lead to the abnormal activation of the PI3K pathway, while the abnormalities of PTEN and TP53 genes could lead to the release of pathway inhibition. The final results could be that the regulation of the cells is out of control and abnormal proliferation of the cells occurs. The statistics have shown that the mutation rates of PIK3CA and PTEN in CRCs are ~12% and ~6%, respectively; mutations of PIK3CA are common in exon 9 and exon 20, and mainly occur in colon-cancer patients [77–79]. However, the frequency of TP53 mutations in CRCs is still unclear.

Increasing evidence has shown that the efficacy of anti-EGFR therapy will be greatly reduced in patients harboring the above mutations or expressing low PTEN and TP53 levels [80–87]. Although the differences in the clinical outcomes between patients with single exon 9 mutations of PIK3CA and patients with single exon 20 mutations of PIK3CA are not significant, when there are double mutations in patients, the clinical outcomes will be worse [85, 86]. Therefore, although there is no guideline to recommend using the genotypes of PIK3CA, PTEN, and TP53 to guide the choice of treatment regimens in CRCs, the role of these three genotypes or the expression levels of PTEN and p53 in anti-EGFR mAbs resistance should not be ignored.

The PI3K pathway has two major targets: PI3K and mTOR (Figure 1). At present, mTOR inhibitors such as everolimus are mainly used in the targeted therapy of advanced renal-cell carcinoma; they have not been recommended for the treatment of mCRC [88, 89]. Although they have shown curative efficacy in mCRCs, their safety and effectiveness still need to be further studied. At the same time, the study and development of PI3K inhibitors have achieved preliminary success: a number of PI3K inhibitors such as alpelisib (BYL719), duvelisib, copanlisib, and idelalisib have been approved for the targeted therapy of PIK3CA-mutant head and neck tumors, breast cancer, or lymphoma; besides, dactolisib (BEZ235, dual PI3K/mTOR inhibitor), PI-103, buparlisib (BKM120), and so on have been approved for the clinical trials of solid tumors. However, similar to mTOR inhibitors, there are only a few studies that relate to mCRCs. A phase Ib dose-escalation study has shown that the combined treatment of encorafenib plus cetuximab and alpelisib is tolerable and provides promising clinical activity in the difficult-to-treat patient population with BRAF-mutant mCRC [90]. The results of some ongoing clinical trials that aim to combine PI3K or mTOR inhibitors with other targeted agents (NCT01719380, NCT01337765, and NCT01591421) to block the PI3K and MAPK pathways at the same time and eliminate drug resistance in mCRCs deserve our exploration. In addition, because PTEN mutations are also often accompanied by MSI-H [79], researchers are trying to use one PI3K inhibitor in combination with nivolumab to treat patients (NCT03711058, NCT03735628).

**Abnormal activations between the parallel pathways**

**Amplifications or mutations of HER2 and HER3**

HER2 and HER3 are two distinct members of the ERBB/HER protein family; they are both non-autonomous. HER2 (ERBB2/neu) does not bind with EGF-like ligands, but it acts as the preferred heterodimer partner of the other three members of the ERBB family [91]. Heterodimers such as EGFR/HER2 have higher affinity and specificity for the ligands [92]. Meanwhile, HER2 can bind more phosphorysine-binding protein than other receptors [93]. In addition, the internalization process of dimers-containing HER2 will be slower and the receptors will return to the cell surface more effectively to receive the second stimulation [94, 95]. While the kinase activity of HER3 (ERBB3) is defective, it works by forming heterodimeric complexes with other receptors that are capable of generating potent cellular signals and then recruiting PI3K to six distinct sites in heterodimeric complexes, thus strongly activating PI3K in a RAS-independent manner [96]. Therefore, both HER2 and HER3 amplifications (also HER2 and HER3 overexpression) and mutations could increase the malignancy degree of the tumors through these mechanisms.

Although the amplification and mutations of HER2 are most common in breast cancer, research over the past decade has shown that 3%–5% of CRCs harbor primary overexpression of HER2 or HER2 mutations, and the prevalence is higher in RAS and BRAF WT CRCs [reported in about 5%–14%] (according to HERACLES criteria: immunohistochemistry 3+ or 2+ in >50% cells confirmed by fluorescence in situ hybridization) [97–100]. Meanwhile, 2% of patients harbor acquired mutations or amplification of HER2 gene after receiving anti-EGFR therapy, and their primary tumors mostly focus in the left colon and rectum and are RAS WT [101, 102]. However, the prevalence of HER3 amplification and mutations seems higher than that of HER2. According to the statistics, 5.7%–11% of CRCs harbor HER3 mutations [100, 103] and 51%–70% of CRCs show the overexpression of HER3 protein, but the evaluated criteria of HER3 overexpression are different [104, 105]. Since 2011, emerging studies have reported that the resistance to anti-EGFR mAbs is related to the primary or acquired amplification and mutations of HER2 [106–110], but the evidence does not support a prognostic role for HER2 overexpression [111]. As for HER3, although the study of the relationship between HER3 and anti-EGFR mAbs resistance is relatively later and smaller than that of HER2, much evidence has also revealed that abnormalities in HER3 could also reduce the efficacy of anti-EGFR therapy in the treatment of mCRCs [104, 112–114]. These findings reveal a novel mechanism of resistance to anti-EGFR mAbs: the abnormalities of HER2 and HER3. Therefore, HER2 has become a new target of mCRC genotyping and treatment up to now (Table 1). However, the diagnostic criteria for the overexpression of HER3, more authoritative evidence of the relationship between HER3 and the resistance to anti-EGFR mAbs, and HER3-targeted agents approved for markets are all required to render HER3 as a new genotyping and treatment target of mCRC.

HER2-targeted agents that have been approved for markets include the mAbs trastuzumab and pertuzumab, the inhibitors
of ERBB family tyrosine kinase, neratinib, afatinib, and pyrotinib, the conjugated agents trastuzumab-entansine (T-DM1) and trastuzumab-hyaluronidase-oysk, and the dimerization inhibitors of EGFR/HER2 tyrosine kinase lapatinib (Table 2); they are mainly approved for the treatment of breast cancer. A number of multicenter, open phase II clinical trials have shown that trastuzumab plus lapatinib has good efficacy and tolerability in the treatment of patients who are HER2-positive [98, 115]; therefore, the latest NCCN guideline recommends that trastuzumab plus pertuzumab or lapatinib can be used in RAS WT mCRCs with HER2 amplification for the subsequent therapy. Although a randomized, open-label phase II trial of afatinib vs cetuximab in mCRCs has shown that the efficacy of afatinib is modest in patients with KRAS mutations [116], the use of tyrosine kinase inhibitors in mCRCs with or without HER2 mutations or amplification needs more evidence to support it [108]. The conjugated agents ado-trastuzumab-emtansine (T-DM1) and trastuzumab-hyaluronidase-oysk were approved for markets by the USA, Europe, and China for the treatment of HER2-positive breast cancer in 2019, but they have been rarely tried for mCRC. At present, many related clinical trials have been started (NCT03418558, NCT03225957, NCT03457896, NCT01919879, and NCT03843749). However, no HER3-targeted agent has been approved for markets. HER3-specific or bispecific inhibitors that have been approved for clinical trials in the past 5 years mainly include the following types: the humanized HER3 mAbs lumretuzumab, ISU104, and CDX-3379; the dual-action HER3/EGFR mAbs zenocutuzumab, duligotuzumab (MEHD7945A); and the novel conjugated agent U3-1402 (Table 2); however, the efficacy of these agents in the trials is inconsistent and controversial [117–121]. In addition, both the efficacy and the tolerability of HER2/HER3-targeted drugs plus EGFR mAbs are also worthy of exploration.

Table 2. Abnormal activations between the parallel pathways and the potential regimens

| Receptor/ligand | Functions | Change | Main targeted drugs (for market* or clinic trial) |
|----------------|-----------|--------|-----------------------------------------------|
| HER2           | As the preferred heterodimeric partner of the other three ERBB members and amplifying the cascades | Amplification or mutations | • HER2 antibodies: trastuzumab*, pertuzumab<br>• Kinase inhibitors: neratinib*, afatinib*, pyrotinib*<br>Specific kinase inhibitors: lapatinib; tucatinib<br>Conjugated drugs: ado-trastuzumab-emtansine*, trastuzumab/hyaluronidase-oysk*<br>HER3 antibodies: lumretuzumab, ISU104, CDX-3379 | |
| HER3           | As the heterodimeric partner of the growth factor receptors and directly activating PI3K signaling in a RAS-independent way | Amplification or mutations | Dual HER3/EGFR antibodies: zenocutuzumab, duligotuzumab (MEHD7945A)<br>Kinase inhibitors: neratinib*, afatinib*, pyrotinib*<br>Conjugated drugs: U3-1402 | |
| HGFR           | Binding to HGF and activating multiple signal-transduction pathways, such as the MAPK pathway, the PI3K pathway, the NF-κB pathway, and the STAT3 pathway | Amplification | Dual EGFR/HGFR antibodies: Sym-015, JNJ-61186327, LY3164530, EMBO1<br>Kinase inhibitors: crizotinib*, cabozantinib*<br>Specific inhibitors: volitinib<br>Conjugated drugs: ABV-399, SHR-A1403 | |
| IGF1           | Binding to IGF1R and activating multiple growth-related signal-transduction pathways that include the MAPK pathway and the PI3K pathway | Amplification | IGF1R antibodies: temprotumumab*, ganitumab, dalotuzumab, IMC-A12, dalotuzumab | |
| IGF2           | Similar to IGF1 | Amplification | IGF2 antibodies | |

HGF, hepatocyte growth factor; HGFR, hepatocyte growth factor receptor; PI3K, phosphatidylinositol 3-kinase; MAPK, mitogen-activated protein kinase; STAT3, transducer and activator of transcription 3; IGF, insulin-like growth factor; IGF1R, insulin-like growth factor 1 receptor; IR, insulin receptor; *have been approved for markets.

Amplification of MET

Hepatocyte growth factor receptor (HGFR, also known as MET tyrosine kinase receptor) is encoded by proto-oncogene MET [122], whereas its ligand HGF is produced by the surrounding fibroblasts and plays a biological effect by paracrine on the adjacent cells that express HGFR [19, 123]. The binding of HGF and HGFR leads to efficient activation of downstream signal-transduction pathways that include MAPK cascades, PI3K/Akt axis, the nuclear factor-κB inhibitor-κB (NF-κB)–nuclear factor-κB (NF-κB) complex, and the signal transducer and activator of transcription 3 (STAT3) [124]; besides, HGFR can also be coupled with HER3 to form a heterodimer under certain conditions [125]. Therefore, MET can not only participate in the occurrence of various morphological events both in the embryo and adulthood, but also drive the malignant progress or drug resistance of a variety of tumors, including CRC.

Currently, both preclinical and clinical studies have shown that MET abnormalities in CRC mainly manifest in gene amplification that can resist the inhibition effect of anti-EGFR mAbs on tumors and rarely in mutations [126–128]. Although the incidence of innate MET amplification in CRC patients is only 2%–4% [129, 130], as MET is similar to HER2, some CRCs harbor acquired MET amplification during the process of anti-EGFR therapy [128, 131, 132]. Hence, the failure of anti-EGFR therapy caused by the amplification of MET cannot be ignored.

At present, some non-specific inhibitors of MET tyrosine kinase such as crizotinib and cabozantinib have been approved for the treatment of other cancers, but all of the macromolecule specific agents targeting HGFR/HGF are still at the clinical-trials stage. In recent years, the agents and relative clinical trials that deserve our special attention mainly include specific MET tyrosine kinase volitinib, conjugated drugs ABV-399 and SHR-A1403, and dual-action EGFR/HGFR mAbs Sym-015, JNJ-
Insulin-like growth factor 1 (IGF1) and IGF2 are quite important growth factors in the human body. IGF1 receptor (IGF1R) is the common receptor of IGF1 and IGF2. After binding with them, IGF1R can activate multiple signal-transduction pathways, including the RAS/RAF/MAPK and PI3K/AKT pathway. Therefore, the IGF1R and EGF pathways have a close crosstalk and participate in cell proliferation, differentiation, angiogenesis, apoptosis, and other life processes together [139, 140]. Although the concentration of IGF2 in blood circulation is higher than that of IGF1, IGF1 plays a more important role in tumor progression than IGF2 because it is regulated by growth hormones, is not easily degraded, and has 15 times higher affinity with IGF1R [141, 142].

The evidence from clinical trials has shown that the overexpression of IGF1 can render the obvious resistance to anti-EGFR mAbs in mCRCs [104, 143], whereas patients with IGF2 overexpression only show lower sensitivity and a marginal response to anti-EGFR therapy [144]. However, few studies have reported the relationship between abnormal IGF1R and cancers. An in vitro study has shown that the regimen of anti-EGFR mAb ICR62 plus IGF1R tyrosine kinase inhibitor NVP-AEW541 can synergistically enhance the antitumor effect in some CRC cells [145]. Consequently, IGF1 and IGF1R could be two potential factors for anti-EGFR mAbs resistance.

Since IGF1 and IGF2 both act on IGF1R to play a physiological role, many researchers are focusing on targeting IGF1R. However, there are few breakthroughs in the development of IGF1R-targeted agents. Although an IGF1R mAb temprotumumab has been approved for the treatment of thyroid-associated ophthalmopathy, there is no trial of this agent for cancers. A randomized phase II clinical trial showed that the IGF1R inhibitor IMC-A12 has not shown the expected efficacy in vivo, either alone or combined with cetuximab [146]. A larger-sample-size II/III clinical trial suggested that an IGF1R inhibitor dalotuzumab (MK-0646) plus irinotecan and cetuximab regimen has good tolerability in patients, but there is no significant improvement in OS [147]. The other IGF1R inhibitor ganitumab plus panitumumab also did not improve the OS of mCRCs in a phase IB/II clinical study [133]. A phase I clinical trial confirmed that a dual-action inhibitor linshitib (OSI-906) that targets IGF1R and insulin receptor (IR) at the same time has good anti-tumor activity in vivo [148]; unfortunately, before reaching the maximum tolerated dose, the OSI-906 plus mTOR inhibitor everolimus regimen has not manifested clinical efficacy when being used to treat refractory CRC [149]. At present, although it is feasible to inhibit IGF1R in theory, more clinical evidence and novel targeted agents are needed to support that IGF1R can be a therapeutic target after the emergence of resistance to anti-EGFR mAbs.

Other mechanisms

In addition to the primary or acquired changes in these common targets, some changes in genes or expression levels that have been rarely reported, unknown mutants, or epigenetic instability can also render the patients resistant to anti-EGFR therapy.

Some changes that have been rarely reported include NTRK gene fusion, IRS2 amplification or mutations, FGFR1 and MAP2K1 mutations, and the low expression of the Bcl-2-interacting mediator of cell death (Bim) [28, 150, 151]; the occurrence of unknown mutations mostly relates to dMMR. Previous studies have suggested that dMMR is associated with a good prognosis in early-stage disease and is rare in advanced patients, especially in sporadic CRC [152]. However, the latest research has pointed out that, under the pressure of anti-EGFR drugs, human CRC cells with mismatch repair proficient/microsatellite stable (pMMR/MS) can manifest dMMR phenotype in a variety of ways, including downregulating the expression of mismatch repair (MMR) genes (MLH1, MLH2, and MLH6), homologous recombination genes (BRCA2 and RAD51), and double-strand break repair gene EXO1, and gradually replacing high-fidelity DNA polymerase by low-fidelity DNA polymerases to participate in the DNA-replication process. Under the above integrated mechanisms, the genome of these cells begins to produce a large number of unknown adaptive mutations and MSI-H, which leads to these cells obtaining resistance to EGFR inhibitors. At the same time, the study also pointed out that the resistance to anti-EGFR agents in cancer cells will change from temporary to irreversible as the agents last longer [153], which put forward a new thinking on the choice of the targeted agents and the design of the treatment regimens.

Epigenetic instability includes aberrant DNA methylation, histone modification, chromosome remodeling, and non-coding RNA interference [154]; aberrant methylation phenotypes and microRNA (miRNA) interference are closely related to both the development and the drug resistance of CRC [155].

The data have shown that methylation events are more common than mutations in CRC patients [155, 156]. The whole-genome hypermethylation status of patients is significantly associated with the poor efficacy of anti-EGFR therapy, but many related mechanisms remain unclear [157]. At present, the study of cytosine-phosphate-guanine (CpG) island methylation phenotype (CIMP) is relatively extensive; however, due to lack of a unified definition standard, the prevalence of CIMP is temporarily unknown. Some analyses have shown that CIMP often accompanies BRAF mutations and relates to the low expression of MLH1, AREG, and EREG in CRCs, which may be a major mechanism by which patients are resistant to anti-EGFR mAbs [158, 159]. miRNA is a classification of endogenous small non-coding RNA that can regulate the expression of genes after transcription [160]. Although the study of miRNA interference with respect to resistance to anti-EGFR mAbs started relatively late, its role cannot be ignored due to the reversibility and activity of epigenetics that are similar to the interval drug resistance of mCRCs in clinic [161]. At present, miRNA-199a-5p/miRNA-375, miRNA-100/miRNA-125/miRNA-181a-5p, miRNA-425-5p, and miRNA-31-5p have been reported to be related to anti-EGFR therapy resistance in mCRCs [162–165] and the interference mechanisms of some miRNAs are relatively clear. MiRNA-199a-5p and miRNA-375 target PHLP1, which indirectly activates the PI3K/AKT pathway [162], whereas miRNA-100/miRNA-125/miRNA-181a-5p regulates the Wnt/b-catenin-signaling pathway [163]. The above phenomena of epigenetic instability can
interact with genetic mutations or amplification to mediate the resistance to anti-EGFR therapy in patients.

**Discussion and future perspective**

Anti-EGFR mAbs cetuximab and panitumumab were approved for the treatment of mCRC in 2004 and 2006, respectively; after years of clinical practice, they have been approved for the first-line treatment of KRAS/NRAS/BRAF WT mCRC. Although the OS, objective response rate, and progression-free survival of most patients with KRAS/NRAS/BRAF WT are significantly prolonged or increased after treatment with anti-EGFR mAbs, we have to face the reality that there are still some patients harboring the above genotypes who cannot obtain similar benefits, and even some patients who have experienced the clinical process of changing tumor inhibition to drug resistance and disease progression (Figure 2). This process is extremely complex and highly heterogeneous because there are both innate and acquired factors that contribute to the occurrence of drug resistance and the strategy of combined targeted chemotherapy deserves our attention.

Therefore, evaluation has now become the most important task (Figure 2), which should include the toxicity, the comparative efficacy, the best combination regimens, the timing of medication, and the appropriate efficacy evaluation biomarkers or index. We are looking forward to more positive results in order to improve the clinical efficacy of anti-EGFR therapy in mCRCs.

**Authors' contributions**

All authors contributed to the concept development, determined the search strategy, evaluated the results for inclusion, and provided critical review of the manuscript. Finally, all authors have read and approved the final manuscript.

**Funding**

This work was supported by the National Natural Science Foundation of China [81871994 and 81701834], the Guangdong Natural Science Foundation [2019B151502063], and the Guangdong Science and Technology Planning Program [2019B151502063].

**Acknowledgements**

None

**Conflicts of interest**

The authors declare that they have no competing interests.

**References**

1. Bray F, Ferlay J, Soerjomataram I et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394–424.
2. Ng K, Zhu AX. Targeting the epidermal growth factor receptor in metastatic colorectal cancer. Crit Rev Oncol Hematol 2008;65:8–20.
3. Arnold M, Sierra MS, Laversonne M et al. Global patterns and trends in colorectal cancer incidence and mortality. Gut 2017;66:683–91.
4. 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.
5. Douillard JY, Tejpar S, Vanbeckevoort D et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. N Engl J Med 2013;369:1029–34.
6. Hurwitz H, Fehrenbacher L, Novotny W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.
7. Van Cutsem E, Tabernero J, Lakomy R et al. Addition of afilibrcept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. J Clin Oncol 2012;30:3499–506.
8. 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.
9. Li S, Schmitz KR, Jeffrey PD et al. Structural basis for inhibition of the epidermal growth factor receptor by cetuximab. Cancer Cell 2005;7:301–11.
10. Giardello F, Tortora G. EGFR antagonists in cancer treatment. N Engl J Med 2008;358:1160–74.
11. Kurai J, Chikumi H, Hashimoto K et al. Antibody-dependent cellular cytotoxicity mediated by cetuximab against lung cancer cell lines. Clin Cancer Res 2007;13:1552–61.
12. Tomas A, Futter CE, Eden ER. EGFR receptor trafficking: consequences for signaling and cancer. Trends Cell Biol 2014;24:26–34.
13. Petrelli F, Borgonovo K, Barni S. The predictive role of skin rash with cetuximab and panitumumab in colorectal cancer patients: a systematic review and meta-analysis of published trials. Target Oncol 2013;8:173–81.
14. Stintzing S, Kapun C, Laubender RP et al. Prognostic value of cetuximab-related skin toxicity in metastatic colorectal cancer patients and its correlation with parameters of the epidermal growth factor receptor signal transduction pathway: results from a randomized trial of the GERMAN AIO CRC Study Group. Int J Cancer 2013;132:236–45.
15. Van Cutsem E, Teijpar S, Vanbekevoort D et al. Intrapatient cetuximab dose escalation in metastatic colorectal cancer according to the grade of early skin reactions: the randomized EVEREST study. J Clin Oncol 2012;30:2861–8.
16. Petrelli F, Cabiddu M, Borgonovo K et al. Risk of venous and arterial thromboembolic events associated with anti-EGFR agents: a meta-analysis of randomized clinical trials. Ann Oncol 2012;23:1672–9.
17. Zhang D, Ye J, Xu T et al. Treatment related severe and fatal adverse events with cetuximab in colorectal cancer patients: a meta-analysis. J Chemother 2013;25:170–5.
18. Citri A, Yarden Y. EGF-ERBB signalling: towards the systems level. Nat Rev Mol Cell Biol 2006;7:505–16.
19. Chen WS, Lazar CS, Poenie M et al. Requirement for intrinsic protein tyrosine kinase in the immediate and late actions of the EGF receptor. Nature 1987;328:820–3.
20. Fukuoka M, Wu YL, Thongprasert S et al. Biomarker analyses and final overall survival results from a phase III, randomized, open-label, first-line study of gefitinib versus carboplatin/paclitaxel in clinically selected patients with advanced non-small-cell lung cancer in Asia (IPASS). J Clin Oncol 2011;29:2866–74.
21. Bonomi PD, Gandara D, Hirsch FR et al. Predictive biomarkers for response to EGFR-directed monoclonal antibodies for advanced squamous cell lung cancer. Ann Oncol 2018;29:1701–9.
22. Lindeman NI, Cagle PT, Aisner DL et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. J Thorac Oncol 2018;13:323–58.
23. Chung KY, Shia J, Kemenyi NE et al. Cetuximab shows activity in colorectal cancer patients with tumors that do not express the epidermal growth factor receptor by immunohistochemistry. J Clin Oncol 2005;23:1803–10.
24. Hecht JR, Mitchell E, Neubauer MA et al. Lack of correlation between epidermal growth factor receptor status and response to panitumumab monotherapy in metastatic colorectal cancer. Clin Cancer Res 2010;16:2205–13.
25. Cunningham D, Humbert Y, Siena S et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
26. Moroni M, Veronesi S, Benvenuti S et al. Gene copy number for epidermal growth factor receptor (EGFR) and clinical response to anti-EGFR treatment in colorectal cancer: a cohort study. Lancet Oncol 2005;6:279–86.
27. Sartore-Bianchi A, Moroni M, Veronesi S et al. Epidermal growth factor receptor gene copy number and clinical outcome of metastatic colorectal cancer treated with panitumumab. J Clin Oncol 2007;25:3238–45.
28. Bertotti A, Papp E, Jones S et al. The genomic landscape of response to EGFR blockade in colorectal cancer. Nature 2015;526:263–7.
29. 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.
30. Llovet P, Sastre J, Ortega JS et al. Prognostic value of BRAF, PI3K, PTEN, EGFR copy number, amphiregulin and epiregulin status in patients with KRAS Codon 12 wild-type metastatic colorectal cancer receiving first-line chemotherapy with anti-EGFR therapy. Mol Diagn Ther 2015;19:397–408.
31. Van Emburgh BO, Arena S, Siravegna G et al. Acquired RAS or EGFR mutations and duration of response to EGFR blockade in colorectal cancer. Nat Commun 2016;7:13665.
32. El-Deiry WS, Goldberg RM, Lenz HJ et al. The current state of molecular testing in the treatment of patients with solid tumors, 2019. CA Cancer J Clin 2019;69:305–43.
33. Kambata-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.
34. Jacobs B, De Roock W, Pissuauvaux H et al. Amphiregulin and epiregulin mRNA expression in primary tumors predicts outcome in metastatic colorectal cancer treated with cetuximab. J Clin Oncol 2009;27:5068–74.
35. Seligmann JF, Elliott F, Richman SD et al. Combined epiregulin and amphiregulin expression levels as a predictive
biomarker for panitumumab therapy benefit or lack of benefit in patients with RAS wild-type advanced colorectal cancer. JAMA Oncol 2016;2:633–42.

36. Karnoub AE, Weinberg RA. Ras oncopgenes: split personalities. Nat Rev Mol Cell Biol 2008;9:517–31.

37. Brink M, de Goeij AF, Weijenberg MP et al. K-ras oncop gene mutations in sporadic colorectal cancer in The Netherlands Cohort Study. Carcinogenesis 2003;24:703–10.

38. Peeters M, Price TJ, Cervantes A et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 2010;28:4706–13.

39. Fernandez-Medarde A, Santos E. Ras in cancer and development directions. Genes Cancer 2011;2:34–48.

40. Bos JL. Ras oncogenes in human cancer: a review. J Clin Oncol 1989;7:1809–24.

41. 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.

42. Fakhiri MG. Metastatic colorectal cancer: current state and future directions. J Clin Oncol 2015;33:1809–24.

43. Schirripa M, Cremolini C, Loupakis F et al. Role of NRAS mutations as prognostic and predictive markers in metastatic colorectal cancer. Int J Cancer 2015;136:83–90.

44. Lefèvre 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.

45. Peeters M, Oliner KS, Price TJ et al. Analysis of KRAS/NRAS mutations in a phase III study of panitumumab with FOLFIRI compared with FOLFIRI alone as second-line treatment for metastatic colorectal cancer. Clin Cancer Res 2015;21:5469–79.

46. K-Ras(G12C) inhibitors al-

47. 50. Ostrem JM, Peters U, Sos ML et al. K-Ras(G12C) inhibitors allo-

48. 51. Larder H, Dahabreh IJ, Kanaanoff D et al. Assessment of somatic k-RAS mutations as a mechanism associated with resistance to EGFR-targeted agents: a systematic review and meta-analysis of studies in advanced non-small-cell lung cancer and metastatic colorectal cancer. Lancet Oncol 2008;9:962–72.

52. Ostrem JM, Peters U, Sos ML et al. K-Ras(G12C) inhibitors allo-

53. Hallin J, Engstrom LD, Hargis L et al. The KRAS inhibitor MRTX849 provides insight toward therapeutic susceptibility of KRAS-mutant cancers in mouse models and patients. Cancer Discov 2020;10:54–71.

54. Coelho MA, de Carné Trécassen S, Rana S et al. Oncogenic RAS signaling promotes tumor immunoresistance by stabilizing PD-L1 mRNA. Immunity 2017;47:1083–99.e6.

55. Carbone DP, Ciernik IF, Kelley MJ et al. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol 2005;23:1007–14.

56. Toubai A, Achtar M, Provenzano M et al. Pilot study of mutant ras peptide-based vaccine as an adjuvant treatment in pancreatic and colorectal cancers. Cancer Immunol Immunother 2008;57:1413–20.

57. Carbone DP, Ciernik IF, Kelley MJ et al. Immunization with mutant p53- and K-ras-derived peptides in cancer patients: immune response and clinical outcome. J Clin Oncol 2005;23:1007–14.

58. Gajtens MK, Bakka A, Breivik J et al. Vaccination with mutant ras peptides and induction of T-cell responsiveness in pancreatic carcinoma patients carrying the corresponding ras mutation. Lancet 1995;346:1399–400.

59. Tran E, Robbins PF, Yu VC et al. T-cell transfer therapy targeting mutant KRAS in cancer. N Engl J Med 2016;375:2255–62.

60. Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. Nat Rev Mol Cell Biol 2004;5:875–85.

61. Gonsalves WI, Mahoney MR, Sargent DJ et al. Patient and tumor characteristics and BRAF and KRAS mutations in colon cancer, NCCTG/Alliance N0147. J Natl Cancer Inst 2014;106: dju106.

62. Davies H, Bignell GR, Cox C et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–54.

63. Di Nicolantonio F, Martini M, Molinar F et al. Wild-type BRAF is required for response to panitumumab or cetuximab in patients with RAS and BRAF wild-type metastatic colorectal cancer. J Clin Oncol 2008;26:5705–12.

64. Pietrantonio F, Petrelli F, Coinu A et al. Predictive role of BRAF mutations in patients with advanced colorectal cancer receiving cetuximab and panitumumab: a meta-analysis. Eur J Cancer 2015;51:587–94.

65. 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.

66. Kopetz S, Desai J, Chan E et al. Phase II pilot study of vemurafinib in patients with metastatic BRAF-mutated colorectal cancer. J Clin Oncol 2015;33:4032–8.

67. Prahallad A, Sun C, Huang S et al. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. Nature 2012;483:100–3.

68. 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 vemurafinib. Cancer Discov 2012;2:227–35.
71. Corcoran RB, Andre T, Atreyea CE et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. Cancer Discov 2018;8:428–43.

72. Kopetz S, Grothey A, Yaeger R et al. Encorafenib, binimetinib, and cetuximab in BRAF V600E-mutated colorectal cancer. N Engl J Med 2019;381:1632–43.

73. Van Cutsem E, Huijbers S, Grothey A et al. Binimetinib, encorafenib, and cetuximab triplet therapy for patients with BRAF V600E-mutant metastatic colorectal cancer: safety lead-in results from the phase III BEACON Colorectal Study. J Clin Oncol 2019;37:1460–9.

74. Clancy C, Burke JP, Kalady MF et al. BRAF mutation is associated with distinct clinicopathological characteristics in colorectal cancer: a systematic review and meta-analysis. Colorectal Dis 2013;15:e711–8.

75. Malek M, Kielkowska A, Chessa T et al. PTEN regulates PI(3,4)P2 signaling downstream of Class I PI3K. Mol Cell 2017;68:566–80.e10.

76. Simpson L, Parsons R. PTEN: life as a tumor suppressor. Exp Cell Res 2001;264:29–41.

77. Shen Y, Wang J, Han X et al. Effectors of epidermal growth factor receptor pathway: the genetic profiling ofKRAS, BRAF, PIK3CA, NRAS mutations in colorectal cancer characteristics and personalized medicine. PLoS One 2013;8:e81628.

78. Sukawa Y, Yamamoto H, Nosho K et al. PTEN for anti-EGFR treatment in metastatic colorectal cancer treated with cetuximab, oxaliplatin and UFT. J Cancer Res Clin Oncol 2009;135:1851–7.

79. Oden-Gangloff A, Di Fiore F, Bibeau F et al. PTEN and exon mutation-specific clinicopathologic and molecular associations in colorectal cancer. Clin Cancer Res 2013;19:3285–96.

80. Sartore-Bianchi A, Martini M, Molinari F et al. PIK3CA mutations in colorectal cancer are associated with clinical resistance to EGFR-targeted monoclonal antibodies. Cancer Res 2009;69:1851–7.

81. Razis E, Pentheroudakis G, Rigakos G et al. EGFR gene gain and PTEN protein expression are favorable prognostic factors in patients with KRAS wild-type metastatic colorectal cancer treated with cetuximab. J Cancer Res Clin Oncol 2014;140:737–48.

82. Oden-Gangloff A, Di Fiore F, Bibeau F et al. TP53 mutations predict disease control in metastatic colorectal cancer treated with cetuximab-based chemotherapy. Br J Cancer 2009;100:1330–5.

83. Di Bartolomeo M, Pietrantonio F, Perrone F et al. Lack of KRAS, NRAS, BRAF and TP53 mutations improves outcome of elderly metastatic colorectal cancer patients treated with cetuximab, oxaliplatin and UFT. Target Oncol 2014;9:155–62.

84. Huemer F, Thaler J, Piringer G et al. Sidedness and TP53 mutations impact OS in anti-EGFR but not anti-VEGF treated mCRC—an analysis of the KRAS registry of the AGMT (Arbeitsgemeinschaft Medikamentöse Tumortherapie). BMC Cancer 2018;18:11.

85. Therkildsen C, Bergmann TK, Henriksen-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.

86. Liao X, Morikawa T, Lochhead P et al. Prognostic role of PIK3CA mutation in colorectal cancer: cohort study and literature review. Clin Cancer Res 2012;18:2257–68.

87. Vitiello PP, Cardone C, Martini G et al. Receptor tyrosine kinase-dependent PI3K activation is an escape mechanism to vertical suppression of the EGFR/RAS/MAPK pathway in KRAS-mutated human colorectal cancer cell lines. J Exp Clin Cancer Res 2019;38:41.

88. Cai Z, Ke J, He X et al. Significance of mTOR signaling and its inhibitor against cancer stem-like cells in colorectal cancer. Ann Surg Oncol 2014;21:179–88.

89. Wilpin BM, Ng K, Zhu AX et al. Multicenter phase II study of tivozanib (AV-951) and everolimus (RAD001) for patients with refractory, metastatic colorectal cancer. Oncologist 2013;18:377–8.

90. van Geel R, Lober A, Elez E et al. Phase I dose-escalation study of encorafenib and cetuximab with or without alpelisib in metastatic BRAF-mutant colorectal cancer. Cancer Discov 2017;7:610–9.

91. Tzahar E, Waterman H, Chen X et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 1996;16:5276–87.

92. Worthylake R, Opresko MK, Krasinski V, et al. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. Embo J 1998;17:3385–97.

93. Jones RB, Gandus O, Kral JA et al. A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature 2006;439:168–74.

94. Baulida J, Kraus MH, Alimandi M et al. ErbB receptors other than the epidermal growth factor receptor are endocytosis impaired. J Biol Chem 1996;271:5251–7.

95. Lenferink AE, Pinkas-Kramarski R, van de Poll ML et al. Differential endocytic routing of homo- and hetero-dimeric ErbB tyrosine kinases confers signaling superiority to receptor heterodimers. Embo J 1998;17:3385–97.

96. Black LE, Longo JF, Carroll SL. Mechanisms of receptor tyrosine-protein kinase ErbB-3 (ERBB3) action in human neoplasia. Am J Pathol 2019;185:1898–912.

97. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. Nature 2012;487:330–7.

98. Sartore-Bianchi A, Trusolino L, Martino C et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. Lancet Oncol 2016;17:738–46.

99. Siena S, Sartore-Bianchi A, Marsoni S et al. Targeting the human epidermal growth factor receptor 2 (HER2) oncogene in colorectal cancer. Ann Oncol 2018;29:1108–19.

100. Loree JM, Bailey AM, Johnson AM et al. Molecular landscape of ERBB2/ERBB3 mutated colorectal cancer. J Natl Cancer Inst 2018;110:1409–17.

101. La Salvia A, Lopez-Gomez V, Garcia-Carbonero R. HER2-targeted therapy: an emerging strategy in advanced colorectal cancer. Expert Opin Investig Drugs 2019;28:29–38.

102. Mohan S, Heitzer E, Ulz P et al. Changes in colorectal carcinoma genomes under anti-EGFR therapy identified by whole-genome plasma DNA sequencing. PLoS Genet 2014;10:e1004271.

103. Jaiswal BS, Kijavin NM, Stawiski EW et al. Oncogenic ERBB3 mutations in human cancers. Cancer Cell 2013;23:603–17.

104. Scartozzi M, Giampieri R, Maccaroni E et al. Combined BRAF, EGFR, and MEK inhibition in patients with BRAF(V600E)-mutant colorectal cancer. Cancer Discov 2018;8:428–43.
patients receiving irinotecan–cetuximab. Ann Oncol 2012;23: 1706–12.

105. Lédél F, Hallström M, Ragnhammar P et al. HER3 expression in patients with primary colorectal cancer and corresponding lymph node metastases related to clinical outcome. Eur J Cancer (Oxford, England: 1990) 2014;50:656–62.

106. Ciardiello F, Normanno N. HER2 signaling and resistance to the anti-EGFR monoclonal antibody cetuximab: a further step toward personalized medicine for patients with colorectal cancer. Cancer Discov 2011;1:472–4.

107. Yonesaka K, Zejnllahu K, Okamoto I et al. Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab. Sci Transl Med 2011;3:99ra86.

108. Kavuri SM, Jain N, Galimi F et al. HER2 activating mutations are targets for colorectal cancer treatment. Cancer Discov 2015;5:832–41.

109. Bertotti A, Migliardi G, Galimi F et al. A molecularly annotated platform of patient-derived xenografts (‘xenopatients’) identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. Cancer Discov 2011;1: 508–23.

110. Martin V, Landi L, Molinari F et al. HER2 gene copy number status may influence clinical efficacy to anti-EGFR monoclonal antibodies in metastatic colorectal cancer patients. Br J Cancer 2013;108:668–75.

111. Wu S-W, Ma C-C, Li W-h. Does overexpression of HER-2 correlate with clinicopathological characteristics and prognosis in colorectal cancer? Evidence from a meta-analysis. Diagn Pathol 2015;10:144.

112. Scartozzi M, Mandolesi A, Giampieri R et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol 2019;20:518–30.

113. Seligmann JF, Hatch AJ, Richman SD et al. Association of tumor HER3 messenger RNA expression with panitumumab efficacy in advanced colorectal cancer. JAMA Oncol 2018;4: 564–8.

114. Liévré A, Ouine B, Canet J et al. Protein biomarkers predictive for response to anti-EGFR treatment in RAS wild-type metastatic colorectal carcinoma. Br J Cancer 2017;117:1819–27.

115. Meric-Bernstam F, Hurwitz H, Raghav KPS et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. Lancet Oncol 2019;20:518–30.

116. Hickish T, Cassidy J, Propper D et al. A randomised, open-label phase II trial of afatinib versus cetuximab in patients with metastatic colorectal cancer. Eur J Cancer (Oxford, England: 1990) 2014;50:3136–44.

117. Koganemaru S, Kuboki Y, Koga Y et al. U3-1402, a novel HER3-targeting antibody-drug conjugate, for the treatment of colorectal cancer. Mol Cancer Ther 2019;18:2043–50.

118. Lieu CH, Hidalgo M, Berlin JD et al. A phase Ib dose-escalation study of the safety, tolerability, and pharmacokinetics of cobimetinib and duligotuzumab in patients with previously treated locally advanced or metastatic cancers with mutant KRAS. Oncologist 2017;22:1024–89.

119. Meulendijks D, Jacob W, Martínez-Garcia M et al. First-in-human phase I study of lumretuzumab, a glycoengineered humanized anti-HER3 monoclonal antibody, in patients with metastatic or advanced HER3-positive solid tumors. Clin Cancer Res 2016;22:877–85.

120. Juric D, Dienstmann R, Cervantes A et al. Safety and pharmacokinetics/pharmacodynamics of the first-in-class dual action HER3/EGFR antibody MEHD7945A in locally advanced or metastatic epithelial tumors. Clin Cancer Res 2015;21:2462–70.

121. Hill AG, Findlay MP, Burge ME et al. Phase II study of the dual EGFR/HER3 inhibitor duligotuzumab (MEHD7945A) versus cetuximab in combination with FOLFIRI in second-line wild-type metastatic colorectal cancer. Clin Cancer Res 2018;24: 2276–84.

122. Bottaro DP, Rubin JS, Faletto DL et al. Identification of the hematocyte growth factor receptor as the c-met proto-oncogene product. Science 1991;251:802–4.

123. Stoker M, Gherardi E, Perryman M et al. Scatter factor is a fibroblast-derived modulator of epithelial cell mobility. Nature 1987;327:239–42.

124. Trusolino L, Bertotti A, Comoglio PM. MET signalling: principles and functions in development, organ regeneration and cancer. Nat Rev Mol Cell Biol 2010;11:834–48.

125. Engelman JA, Zejnllahu K, Mitsudomi T et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039–43.

126. Luraghi P, Reato G, Cipriano E et al. MET signalling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. Cancer Res 2014;74:1857–69.

127. Liska D, Chen CT, Bachleitner-Hofmann T et al. HGF rescues colorectal cancer cells from EGFR inhibition via MET activation. Clin Cancer Res 2011;17:472–82.

128. Bardelli A, Corso S, Bertotti A et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2012;2:658–73.

129. Misale S, Di Nicolantonio F, Sartore-Bianchi A et al. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. Cancer Discov 2014;4: 1269–80.

130. Zhang M, Li G, Sun X et al. MET amplification, expression, and exon 14 mutations in colorectal adenocarcinoma. Hum Pathol 2018;77:108–15.

131. Pietrantonio F, Oddo D, Gloghini A et al. MET-driven resistance to dual EGFR and BRAF blockade may be overcome by switching from EGFR to MET inhibition in BRAF-mutated colorectal cancer. Cancer Discov 2016;6:963–71.

132. Pietrantonio F, Vernieri C, Siravegna G et al. Heterogeneity of acquired resistance to anti-EGFR monoclonal antibodies in patients with metastatic colorectal cancer. Clin Cancer Res 2017;23:2144–22.

133. Van Cutsem E, Eng C, Nowara E et al. Randomized phase Ib/II trial of rilotumumab or ganitumab with panitumumab versus panitumumab alone in patients with wild-type KRAS metastatic colorectal cancer. Clin Cancer Res 2014;20:4240–50.

134. Eng C, Bessudo A, Hart LL et al. A randomized, placebo-controlled, phase 1/2 study of tivantinib (ARQ 197) in combination with irinotecan and cetuximab in patients with metastatic colorectal cancer with wild-type KRAS who have received first-line systemic therapy. Int J Cancer 2016;139: 177–86.

135. Bendell JC, Ervin TJ, Gallinsson D et al. Treatment rationale and study design for a randomized, double-blind, placebo-controlled phase II study evaluating onartuzumab (MetMaB) in combination with bevacizumab plus mFOLFOX-6 in patients with previously untreated metastatic colorectal cancer. Clin Colorectal Cancer 2013;12:218–22.

136. Spigel DR, Edelman MJ, O’Byrne K et al. Results from the phase III randomized trial of onartuzumab plus erlotinib versus erlotinib in previously treated stage IIIb or IV non-small-cell lung cancer: METLung. J Clin Oncol 2017;35:412–20.
137. Catenacci DVT, Tebbutt NC, Davidenko I et al. Rilotumumab plus epirubicin, cisplatin, and capicitabine as first-line therapy in advanced MET-positive gastric or gastro-esophageal junction cancer (RILOMET-1): a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Oncol 2017;18: 1467–82.

138. Rimassa L, Assenat E, Peck-Radosavljevic M et al. Tivantinib for second-line treatment of MET-high, advanced hepatocellular carcinoma (METIV-HCC): a final analysis of a phase 3, randomised, placebo-controlled study. Lancet Oncol 2018;19:682–93.

139. Furstenberger G, Senn HJ. Insulin-like growth factors and cancer. Lancet Oncol 2002;3:298–302.

140. Hu YP, Patil SB, Panasiewicz M et al. Heterogeneity of receptor function in colon carcinoma cells determined by cross-talk between type I insulin-like growth factor receptor and epidermal growth factor receptor. Cancer Res 2008;68: 8004–13.

141. Jones JI, Clemmons DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.

142. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 1997;18:801–31.

143. Scartozzi M, Mandolesi A, Giampieri R et al. Insulin-like growth factor 1 expression correlates with clinical outcome and functions. Int J Cancer 2017;140:686–700.

144. Zanella ER, Galimi F, Sassi F et al. IGF2 is an actionable target that identifies a distinct subpopulation of colorectal cancer patients with marginal response to anti-EGFR therapies. Sci Transl Med 2015;7:272ra12.

145. Cunningham MP, Thomas H, Marks C et al. Co-targeting the EGFR and IGF-IR with anti-EGFR monoclonal antibody ICR62 and the IGF-IR tyrosine kinase inhibitor NVP-AEW541 in colorectal cancer cells. Int J Oncol 2008;33:1107–13.

146. Reidy Dl, Vakiani E, Fakih MG et al. Randomized, phase II study of the insulin-like growth factor-1 receptor inhibitor IMC-A12, with or without cetuximab, in patients with cetuximab- or panitumumab-refractory metastatic colorectal cancer. J Clin Oncol 2010;28:4240–6.

147. Scalfani F, Kim TY, Cunningham D et al. A randomized phase II/III study of dalotuzumab in combination with cetuximab and irinotecan in chemorefractory, KRAS wild-type, metastatic colorectal cancer. J Natl Cancer Inst 2015;107: djv258.

148. Puzanov I, Lindsay CR, Goff L et al. A phase I study of continuous oral dosing of OSI-906, a dual inhibitor of insulin-like growth factor-1 and insulin receptors, in patients with advanced solid tumors. Clin Cancer Res 2015;21:701–11.

149. Bendell JC, Jones SF, Hart L et al. A phase Ib study of linsitinib (OSI-906), a dual inhibitor of IGF-1R and IR tyrosine kinase, in combination with everolimus as treatment for patients with refractory metastatic colorectal cancer. Invest New Drugs 2015;33:187–93.

150. Lian T, Li C, Wang H. Trametinib in the treatment of multiple malignancies harboring MEK1 mutations. Cancer Treat Rev 2019;81:101907.

151. Marzi L, Combes E, Vié N et al. FOXO3a and the MAPK p38 are activated by cetuximab to induce cell death and inhibit cell proliferation and their expression predicts cetuximab efficacy in colorectal cancer. Br J Cancer 2016;115:1223–33.

152. Koopman M, Kortman GA, Mekenkamp L et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer 2009;100:266–73.

153. Russo M, Crisafiulli G, Sogari A, et al. Adaptive mutation of colorectal cancers in response to targeted therapies. Science 2019;366:1473–80.

154. Esteller M. Epigenetics in cancer. N Engl J Med 2008;358: 1148–59.

155. Okugawa Y, Grady WM, Goel A. Epigenetic alterations in colorectal cancer: emerging biomarkers. Gastroenterology 2015; 149:1204–25.e12.

156. Lao VV, Grady WM. Epigenetics and colorectal cancer. Nat Rev Gastroenterol Hepatol 2011;8:686–700.

157. Ouchi K, Takahashi S, Yamada Y et al. DNA methylation status as a biomarker of anti-epidermal growth factor receptor treatment for metastatic colorectal cancer. Cancer Sci 2015;106:1722–9.

158. Weisenberger DJ, Siegmund KD, Campan M et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. Nat Genet 2006;38:787–93.

159. Lee MS, McGuffey EJ, Morris JS et al. Association of CpG island methylator phenotype and EREG/AREG methylation and expression in colorectal cancer. Br J Cancer 2016;114: 1352–61.

160. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.

161. Salgia R, Kulkarni P. The genetic/non-genetic duality of drug ‘resistance’ in cancer. Trends Cancer 2018;4:110–8.

162. Mussnich P, Rosa R, Bianco R et al. MicroRNA-425-5p expression in colorectal cancer: emerging biomarkers. J Clin Oncol 2008;26:1223–33.

163. Lu Y, Zhao X, Liu Q et al. lncRNA MIR100HG-derived miR-100 affects colon cancer cell sensitivity to cetuximab by targeting PHLPP1. Exp Opin Ther Targets 2015;19:1017–26.

164. Luo Y, Zhao X, Liu Q et al. IncRNA MIR100HG-derived miR-100 and miR-125b mediate cetuximab resistance via Wnt/beta-catenin signaling. Nat Med 2017;23:1331–41.

165. Igarashi H, Kurihara H, Mitsuhashi K et al. Deficient mismatch repair system in patients with sporadic advanced colorectal cancer. Br J Cancer 2009;100:266–73.

166. Gschwind A, Glise S, Quan P et al. The genetic fingerprint of colorectal cancer. Science 2019;366:1473–80.