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Resistance to targeted therapy and new developments in metastatic colorectal cancer

Tang YL *et al.* Targeted therapy resistance and new development

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Abstract

Colorectal cancer (CRC) is one of the most lethal and common malignancies in the world. Chemotherapy has been the conventional treatment for metastatic CRC (mCRC) [A1] patients. However, the effects of chemotherapy have been unsatisfactory. With the advent of targeted therapy, the survival of patients with CRC have been prolonged. Over the past 20 years, targeted therapy for CRC has achieved substantial progress. However, targeted therapy has the same challenge of drug resistance as chemotherapy. Consequently, exploring the resistance mechanism and finding strategies to address the resistance of targeted therapy, along with searching for novel effective regimens is a constant challenge in the mCRC treatment, and it is also the research hotspot. In this review, we focus on resistance to existing targeted therapies and discuss future developments in mCRC.

Key Words: Colorectal cancer; Targeted treatment; Resistance; New development

Core Tip: Colorectal cancer (CRC) is one of the most lethal and common malignancies in the world. Chemotherapy has long been the mainstay of CRC treatment. However, since chemotherapy is not a specific regimen, it will produce systemic toxicity. Following, with the advent of targeted therapy, the prognosis of CRC has been improved significantly. Although targeted therapy also develop drug resistance, more and more novel targets and combination regimens are being explored over the past 20 years. In this review, we summarized resistance to exiting targeted therapy, and discussed future developments in CRC.

INTRODUCTION

Colorectal cancer (CRC) is the third most prevalent malignancy and the second most lethal cancer worldwide^[1]. Surgery is the primary treatment option and has the potential to be curative, especially for patients in early-stage of disease^[2]. However, most patients suffer from advanced/metastatic tumors, with a five-year overall survival (OS) rate of approximately 13%^[3]. Systemic chemotherapy is the cornerstone of treatment for these patients, resulting in a median OS (mOS) of 17-23 mo^[4-6]. The prognosis of patients with mCRC has improved with the addition of targeted therapies, such as antibodies against epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and tyrosine kinase inhibitors (TKIs)^[7,8].

CRC is a heterogeneous disease with multiple molecular features, requiring individual targeted approach for achieving effective disease control and good survival rates^[9]. This review presents the standard targeted therapy options and corresponding resistance mechanisms, and discuss the promising targeted agents for mCRC.

CURRENT TREATMENT FOR COLORECTAL CANCER

Conventional treatments for CRC include 5-fluorouracil (5-FU)-based chemotherapy including leucovorin, 5-FU, and oxaliplatin (FOLFOX), leucovorin, 5-FU, and irinotecan (FOLFIRI), and capecitabine and oxaliplatin (CAPOX)^[10,11]. CRC chemotherapy has certain limitations due to systemic toxicity, low population-specific selectivity, and unsatisfactory response rate. Targeted therapies, including small-molecule inhibitors and monoclonal antibodies are effective approaches following chemotherapy. The antiangiogenic drugs bevacizumab and anti-EGFR regimens have been successively approved by the Food and Drug Administration (FDA) for both first- and second-line CRC treatment^[12-15]. In the Biomarkers of Nutrition for Development (BOND) trial, cetuximab showed clinically significant activity to improve the response rate and progression-free survival (PFS) in patients with irinotecan-refractory colorectal cancer; the most frequently observed adverse events were diarrhea, asthenia, and acne-like rash^[12]. Panitumumab has a lower risk of hypersensitivity reactions than cetuximab, because panitumumab is a fully humanized antibody unlike others, which are murine-human chimeric antibodies^[16]. The efficacy and safety of panitumumab plus fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) has been proven in the PRIME trial^[15,17]. Randomized controlled trials have shown that bevacizumab with chemotherapy could significantly increase OS and PFS in patients with CRC^[14,18,19], and both patients with Kirsten rat sarcoma 2 viral oncogene homolog (KRAS) mutations and those with the wild-type genotype could benefit from bevacizumab^[20,21]. Besides, bevacizumab is the only antiangiogenic drug approved for the first-line treatment of CRC; some other novel anti-VEGF drugs have shown favorable results in second-line treatment of CRC, such as aflibercept and ramucirumab^[22-24]. The main toxic effects of bevacizumab are cardiotoxicity, including hypertension and bradycardia. In addition, TKIs, including

regorafenib and fruquintinib, were also options for CRC patients, which have been approved for third-line treatment^[25,26]. TAS-102 is a novel oral drug that is combination of trifluridine and tipiracil. Trifluridine is a cytotoxic nucleic acid analog that leads to DNA dysfunction, whereas tipiracil is a thymidine phosphorylase that inhibits enzyme degradation.

RESISTANCE TO TARGETED THERAPY IN CRC

The EGFR pathway

EGFR is a member of ERBB (erythroblastosis oncogene B)/HER (human EGFR) family. Overexpression of EGFR has been detected in 25%-77% of CRCs^[27]. Notably, subgroup analysis from the CRYSTAL and PRIME trials revealed that anti-EGFR therapy only benefited the population with wild-type (WT) RAS mutation^[13,15]. Patients with BRAF mutations failed to respond to anti-EGFR therapy³⁶ and BRAF mutations were independent of RAS mutations. Therefore, the European Society of Medical Oncology (ESMO)¹⁹ and National Comprehensive Cancer Network (NCCN) guidelines recommend anti-EGFR therapy¹⁰ only for patients with BRAF-WT and RAS-WT. Interestingly, left-sided CRC expressed more EGFR than right-sided CRC. The American Society of Clinical Oncology⁸ recommends that populations with left-sided tumors⁵⁷ vs right-sided tumors seem to benefit more from anti-EGFR therapy and are associated with better prognosis^[28]. It can be seen that not all patients are suitable for anti-EGFR therapy, and even though patients respond to it, resistance develops in 3-12 mo^[29,30]. Gene mutations downstream of the EGFR signaling pathways⁷² are the main causes of resistance to anti-EGFR therapy, including RAS/RAF/MEK and PI3K/AKT/mTOR^[31-33]. In addition, the activation¹ of compensatory pathways of EGFR, such as ERBB2 and MET, is also the reason for the drug resistance to anti-EGFR^[34-36]. In

addition to the intrinsic mechanisms mentioned above, microenvironmental plasticity also conferred cetuximab and panitumumab resistance^[37].

The VEGF pathway

Angiogenesis is essential for tumor initiation, development, and metastasis. Overexpression of the VEGF ligand family and its binding to tyrosine kinase receptors (VEGFR-1, VEGFR-2, and VEGFR-3) result in endothelial cell growth, and migration^[38]. The activation of alternative signaling pathways and production of angiogenesis-related proteins may cause resistance to antiangiogenic therapy. Investigations demonstrated that both patients with KRAS-mutated-type (MT) and those with KRAS-wild-type (WT) could benefit from bevacizumab, which was different from cetuximab^[20,21,39]. Placental growth factor is a pivotal indicator of anti-VEGF resistance, which is part of the reason aflibercept is more effective than bevacizumab in xenograft models^[40,41]. In addition, abnormal increases in angiopoietin-2 have been observed in many types of tumors, including CRC, and have been shown to be associated with resistance to bevacizumab^[42-44]. Activation of the fibroblast growth factor-1 (FGF)/FGFR pathway has the ability to promote cell survival and migration in both normal and malignant tissues, which is also seen in anti-VEGF resistant population^[45,46]. The mechanism most closely associated with the loss of anti-VEGF effectiveness is compensatory stimulation of the c-MET pathway, in which case, single-agent c-MET suppression might be beneficial^[47].

NEW DEVELOPMENTS IN COLORECTAL CANCER

Overcoming anti-EGFR therapy resistance

In general, strategies ¹ to overcome resistance to anti-EGFR regimens include the following: novel EGFR-targeted drugs, multi-targeted regimen combinations, metabolic regulators, and immunotherapy.

EGFR ligands and EGFR: The expression of EGFR ligands potentially correlates with efficacy of anti-EGFR therapy^[48,49]. ¹ Thus, the development of new mAbs [A2] that can target different EGFR epitopes promises to reverse anti-EGFR resistance. Among different EGFR somatic sequence changes located in the extracellular domains (ECDs), S492R affects cetuximab binding, but does not interrupt panitumumab binding, suggesting a ³ rechallenge with panitumumab in patients resistant to cetuximab and developing an S492R mutation^[50]. MM-151, a monoclonal antibody that engages multiple epitope [A3] s on EGFR, interferes with EGFR signalling and suppresses tumor activity in a preclinical study^[51]. Sym004 is a combination of two distinct anti-EGFR mAbs and showed significant superiority in suppressing phosphorylation of EGFR ligand and inhibiting EGFR downstream pathways in a tumor xenograft model^[52,53]. ⁸ A multicenter, phase II clinical trial explored the ² efficacy of Sym004 in patients with mCRC with secondary resistance to anti-EGFR therapy. Although Sym004 did not improve prognosis compared with the control group (capecitabine, fluorouracil, best supportive care), subgroup analysis demonstrated that patients without EGFR ECD mutations had benefitted in OS with Sym004 therapy^[54]. Nectinmumab is another ¹ approved EGFR antibody that can integrate with S468R, the most common ³² cetuximab resistance substitution^[55]. Nectinmumab plus mFOLFOX6 showed favorable survival with manageable toxicity in the first-line treatment of mCRC patients^[56].

Gene mutations in downstream pathways

Targeting RAS mutant CRC: RAS mutations present in nearly half of CRC patients usually occur in KRAS, NRAS and HRAS^[33, 57]. KRAS is the most frequent mutation (40%), predominantly in exon 2, codons 12 and 13, and less commonly in exons 3 and 4^[57-59]. Although RAS mutations are negative predictive markers of anti-EGFR treatment activity and are associated with poor survival outcomes, data have demonstrated⁵⁵ that not all KRAS-mutated patients develop resistance to anti-EGFR treatment^[60]. It is unclear whether patients with KRAS^{G13D} gene mutations^[A4] develop drug resistance^[61-63].

RAS direct inhibitors: RAS proteins are small guanosine triphosphate (GTP) phosphatases (GTPases) and are turned on when GTP-bound^[64]. In the activated state, RAS alters its conformation and activates downstream pathways to promote cell proliferation, migration, and survival. This is precisely because of³ the high affinity of RAS for GTP/GDP and the lack of suitable small molecules targeting known allosteric sites^[65]. Among the most common RAS mutations, KRAS^{G12C} possesses a unique near-WT intrinsic GTPase activity and has recently been demonstrated to be druggable^[66,67]. Sotorasib (AMG 510) is the first drug tested in clinical trials, which specifically and irreversibly inhibits KRAS^{G12C}. The anticancer activity of sotorasib in patients harboring the KRAS^{G12C} mutation has been demonstrated² in a phase I trial. In the subgroup with colorectal cancer, 3 of 42 (7.1%) patients achieved⁵ partial response (PR), 28 (66.7%) experienced stable disease (SD), and the mPFS was 4.0 mo^[67,68]. Adagrasib (MRTX849) is another covalent KRAS^{G12C} inhibitor that achieves an overall³⁹ response rate (ORR) and disease control rate (DCR) of 17% (3/18) and 94% (17/18),⁸ respectively, in patients with KRAS^{G12C} CRC^[69]. In addition, a phase III trial assessed the effect of adagrasib⁷³ plus cetuximab as

second-line treatment for KRAS^{G12C} MT mCRC^[70]. Regrettably, KRAS^{G12C} is found in only 4% of CRCs, and KRAS^{G12D} is the most common type of CRC; thus, developing a specific effective inhibitor is of great significance in clinical practice^[71]. The development of inhibitors directly targeting KRAS^{G12D} is much more difficult than that targeting KRAS^{G12C} because KRAS^{G12D} lacks cysteine, which can covalently bind to small molecular drugs. MRTX1133 is the first noncovalent and specific inhibitor of KRAS^{G12D} with preclinical data. The binding activity and selectivity of MRTX1133 were improved by optimizing the 2, 4, and 7 positions of pyrido[4,3-d] pyrimidine based on adagrasib. In the mouse models of xenograft tumors, MRTX1133 ⁵⁹has been shown to inhibit KRAS signaling and exhibit robust antitumor^[A5] activity^[72]. In addition, Revolution Medicines have developed a covalent KRAS^{G12D} inhibitor that targets the activated state of KRAS. RM-036 forms a tri-complex with chaperone cyclophilin A and the on-state of KRAS^{G12D} and can selectively bind to aspartate of KRAS^{G12D} mutants, effectively inhibiting signal transduction. Treatment with RM-036 resulted in dramatic tumor regression and a complete response in several KRAS^{G12D} mutant xenograft models^[73]. Overall, targeted therapies for KRAS^{G12D} are in the early stages of research, and more clinical trials are needed. Recently, a pan-KRAS probe compound, BI-panKRAS3, demonstrated antitumor efficacy in colorectal cancer (CRC) models with KRAS^{G12D} and KRAS^{G13D} mutations^[74].

Targeting the downstream pathways: MAPK pathway: Inhibition of MAPK effectors is another strategy targeting RAS-MT CRC^[75]. Trametinib, binimetinib, and cobimetinib, which are MEK inhibitors (MEKi), prevent MEK phosphorylation of ERK1/2, thereby preventing dimerization and nuclear translocation^[76]. However, these drugs alone did not show favorable results in this subset of the population^[77-79]. Several studies have focused on simultaneous use of inhibitors of MEK and upstream RTK, such as

combination of MEKi with anti-EGFR drugs^[80,81]. Results from both ¹³ *in vitro* and *in vivo* studies suggest that the combination of cetuximab and trametinib can induce KRAS-MT cell death^[82-85]. Moreover, activation of the MAPK pathway may dysregulate the cell cycle *via* the cyclin-dependent kinase (CDK) pathway. Palbociclib, a CDK4/6 inhibitor, showed limited antitumor activity when used alone (0% ORR and 33% DCR)^[63]. However, the combination of binimetinib and palbociclib exhibited synergistic suppression of cell growth *in vivo* trials^[86]. The efficacy of palbociclib and another CDK4/6 inhibitor, trametinib, was evaluated in a phase Ib study (NCT02065063). A case reported that a CRC patient with KRAS-MT achieved PR for up to 10.8 mo with this combination regimen^[87].

Immunotherapy combination: KRAS mutations might promote cancer immune escape mechanisms, and they are also relevant to ¹³ programmed cell death protein 1 (PD-1) and ⁴⁵ decrease the expression of programmed death-ligand 1 (PD-L1) in microsatellite instability (MSI) CRC^[88]. In preclinical models, sotorasib plus checkpoint inhibitor therapy (immune checkpoint inhibitors, ICIs) increased T cell infiltration and activation, thereby forming a tumor microenvironment that was highly sensitive to immune-checkpoint inhibition^[89]. Similar results were observed with the combination of adagrasib and anti-PD-L^[90].

Targeting RAS through metabolic pathways: KRAS mutations can drive metabolic reprogramming by increasing glucose uptake and protein content and exhibit a Warburg effect phenotype^[91]. Fatty acid synthase (FASN) regulates lipid synthesis and is often upregulated in KRAS MT tumors. A phase I trial explored the pharmacodynamic effects of TVB-2640 (a FASN inhibitor) in patients with resectable tumors (NCT02980029)^[92]. Interestingly, as the first-line treatment for type 2 diabetes mellitus, metformin may have a chemo-preventive role in CRC^[93]. In preclinical studies, metformin showed a synergistic effect with oxaliplatin in the *in vitro*

models of colon cancer^[94]. However, ²⁵ the effect of metformin on the survival of patients receiving chemotherapy after resection is still inconclusive^[95-98]. Additional evidence from prospective randomized controlled trials is needed. It has been reported that cultured CRC cells with KRAS mutations induce energy crisis and cell death when exposed to high doses of vitamin C (AA, vitamin C)^[99]. The favorable safety and preliminary efficacy of AA plus mFOLFOX6/FOLFIRI (irinotecan, 5-fluoruracil, and leucovorin) in mCRC has been proven in a ³⁰ phase I study. Relevant phase II and III studies are ongoing^[100].

Targeting BRAF mutant CRC: BRAF mutations occur ⁵³ in approximately 12% of patients with CRC, and more than 95% of these mutations manifest as V600E substitution^[101-104]. ¹⁰ Patients with BRAF-MT CRC are more common in women and in people diagnosed at an old age, and the primary tumors mainly occur on the right side and in advanced stages, and most are mucinous adenocarcinomas. Moreover, BRAF-MT CRC mainly develops peritoneal and lymph node metastasis^[105-107]. In addition, more than ³ 50% of BRAF V600E mutant CRC have a high MSI (MSI-H) status^[108,109]. Colorectal cancers harboring these features are frequently sporadic and exhibit extensive DNA methylation of CpG islands^[110,111]. ³ In contrast, MSI status in the absence of BRAF V600E mutation is related to Lynch syndrome^[112]. The mOS of patients with BRAF-MT is approximately 11 mo, which is lower than that of patients with BRAF-WT^[106,107]. Notably, non-V600E BRAF is defined as a clinically special subgroup with a good prognosis, which occurs in 2.2% of all patients with CRC^[107]. Several cases have provided evidence that patients with a non-V600 BRAF mutation can respond to EGFR inhibitors^[113, 114].

The NCCN guidelines recommend combination chemotherapy as the foundation of therapy for patients with BRAF-mutated CRC^[8]. The Triplet plus bevacizumab (TRIBE) study compared the effectiveness and safety of FOLFIRI plus bevacizumab vs FOLFOXIRI plus bevacizumab in untreated patients with mCRC. While 16 patients received FOLFOXIRI plus bevacizumab, 12 patients received FOLFIRI plus bevacizumab, with a median OS of 19.0 mo and 10.7 mo respectively^[115]. However, these promising survival data were not confirmed in the TRIBE phase III trial^[116]. In addition, a meta-analysis indicated that FOLFOXIRI with bevacizumab failed to show superiority compared to doublet combination plus bevacizumab in patients with BRAF-mutant tumors^[117]. Therefore, there is insufficient evidence to suggest that triplet combination regimens are better than doublet chemotherapy as first-line treatment for BRAF V600E-mutated CRC.

Monotherapy strategies: Vemurafenib is an oral inhibitor of BRAF V600 kinase, approved by the FDA for metastatic melanoma patients, with an effective rate of 77.1%^[118,119]. Nevertheless, vemurafenib, as a single agent showed limited activity in BRAF-MT mCRC, with a response rate of 5%^[120]. This result illustrates that BRAF activation in mCRC is more intricate and heterogeneous than that in melanoma. The activity of BRAF inhibitors may cause feedback signaling via reactivation of EGFR, whereas melanoma cells express low levels of EGFR^[121,122].

Concurrent blockade of BRAF and EGFR: Several studies have shown that EGFR inhibitors combined with BRAF inhibitors could be an effective strategy^[122,123]. Rona et. al assessed the efficacy of vemurafenib combined with panitumumab in 15 patients with chemo-resistant BRAF-MT mCRC. The results of this trial included 10 tumor regressions and two stable diseases lasting over 6 mo. Additional studies exploring dual blockade of BRAF and EGFR showed response rates of 10%-39%^[124]. Interestingly, adding

irinotecan to the two-drug regimen can further inhibit tumor growth.²⁹ In a phase I trial (NCT01787500), patients with pretreated BRAF-MT mCRC achieved sustained disease control after receiving this triple combination therapy, and the mPFS was 7.7 mo^[125]. The SWOG1406 phase III trial recruited 160 patients with BRAF-MT mCRC and randomly assigned them to two treatment groups: 54 patients received irinotecan, cetuximab, and vemurafenib, while the others received cetuximab and irinotecan. The results showed that the ORR and DCR increased by 17% and 65%, respectively, with the addition of vemurafenib^[126]. Accordingly, the 2018 NCCN guidelines recommended that mCRC patients with BRAF-MT could choose three-drug combination regimens: irinotecan, anti-EGFR therapy, and anti-BRAF therapy (vemurafenib)^[127]. Recently, a study found that RNF43 mutations could help prioritize patients with mCRC BRAF-V600E, who are more likely to respond to the anti-EGFR/BRAF combination. Further research is needed to explore the incorporation of this biomarker along with BRAF and MSS/MSI status in routine testing^[128].

Concurrent blockade of BRAF and MEK: The combination of BRAF and MEK inhibitors can also produce potentially favorable antitumor activity by enhancing inhibition of the MAPK pathway^[129,130]. In the phase III BEACON study, 665 BRAF V600E-MT mCRC patients were enrolled and randomly divided in [A6] to three groups: Encorafenib, binimetinib, and cetuximab; encorafenib and cetuximab; and irinotecan plus FOLFIRI or cetuximab (control group). A combination of triplet therapy significantly improved the OS, with an mOS of 9.0 mo. The objective responses of the triplet therapy, doublet therapy, and control groups were 27%, 20%, and 2%, respectively^[131,132]. Based on these results, the FDA approved doublet regimens as second-line treatment for BRAF-MT mCRC therapy, while in Japan, both doublet and triplet regimens are approved^[133]. The ANCHOR study is an ongoing trial to estimate the efficacy of encorafenib, binimetinib, and cetuximab for BRAF-MT mCRC with previously untreated patients and is

also the first prospective study using BRAF inhibitor regimens as the first-line therapy^[134]. Dabrafenib is another selective BRAF inhibitor competing against ATP^[135]. Ryan *et al*^[64] [A7] tested dabrafenib plus trametinib in 43 patients with BRAF V600-mutated CRC, five patients (12%) achieved partial response, and 24 patients (56%) achieved stable disease^[136].

PI3K/AKT activation and PI3K/AKT inhibitors: PIK3CA encodes the p110 α catalytic subunit of phosphatidylinositol 3-kinase (PI3K), found in 10%-20% of CRC patients^[31]. PIK3CA mutation and abnormal AKT/mTOR activation leads to reversal of EGFR-blocking effects in CRC. PIK3CA mutations usually occur in exons 9 and 20, and exon 20 mutations are associated with worse prognosis in KRAS-WT mCRC patients treated with cetuximab, whereas exon 9 mutations did not affect survival^[31,137]. Experimental data suggest that aspirin suppresses prostaglandin-endoperoxide synthase 2 and downregulates PI3K signaling activity^[138]. Low-dose aspirin had a significant interactive effect on survival among patients with PIK3CA-MT CRC but not among patients with PIK3CA-WT^[139]. This observation requires a prospective evaluation. PTEN (phosphatase and tensin homolog) is a negative modifier of the PI3K/AKT pathway, detected in 20%-40% of mCRC cases, the loss of which results in tumor growth by activating PI3K/AKT^[140]. Tumors without mutations in KRAS, NRAS, BRAF, and PIK3CA are called “quadruple negative” CRCs, responding best to anti-EGFR therapy^[80].

Compensatory feedback loop signaling

HER-2 amplification/overexpression: Amplification of the HER2 gene, which induces excessive PI3KCA/AKT/mTOR signaling, occurred in 5%-7% of CRC patients, mostly in RAS wild-type tumors^[36,141,142]. The incidence of HER2 mutations was not related to patient age, sex, tumor stage or anatomical location^[143]. However, ¹⁶ genomic profiling studies have reported that HER2 mutations are more common in high tumor mutation burden or MSI-H tumors^[144,145].

Early clinical studies evaluating HER2 inhibitors with chemotherapy were terminated because of poor accrual^[146,147]. It has been speculated that the low frequency of HER2 expression in CRC restricts the application of HER2-targeted therapy. Bertotti ²⁹ *et al*^[141] found that patients with HER2 positive were sensitive to dual regimens of trastuzumab and lapatinib but not to either agent alone. HERACLES-A, a multicenter phase II trial, evaluated the efficacy of a ⁴⁴ combination of trastuzumab and lapatinib in mCRC patients with KRAS WT and HER2 overexpression refractory to standard treatment^[148]. HER2 ²⁵ positivity was defined by ²⁴ immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH): tumors with a score of IHC 3+ in more than half of the cells, or with IHC 2+: CEP17 ratio > 2 in more than half of the cells^[149]. The study achieved an ORR of 30% and showed encouraging survival outcomes: ¹⁷ an mPFS of 5 mo and an mOS of 11.5 mo^[148]. Based on these results, trastuzumab and lapatinib regimens were included in the 2019 NCCN Guidelines for CRC, and more clinical research has been triggered to optimize anti-HER2 regimens in this setting^[8]. ³ In the HERACLES-B study, a single-arm phase II trial, patients were treated with pertuzumab and trastuzumab (T-DM1). A total of thirty-one patients were evaluable, of whom 48% had more than four lines of previous therapies. ¹¹ Although the study did not meet its primary endpoint, with an ORR of 9.7%^[150], it demonstrated a similar mPFS to other anti-HER2 regimens with low toxicity and high disease control. In the phase II MyPathway trial, patients also received pertuzumab and

trastuzumab, and the ORR was only 8% in the KRAS-MT population compared to 40% in the KRAS-WT population, suggesting that patients with KRAS-WT mCRC benefited more from the combination of pertuzumab and trastuzumab than those with KRAS-MT mCRC^[151,152]. The efficacy of pertuzumab plus trastuzumab was confirmed in the TRIUMPH trial. This was the first study to define HER2-positive mCRC based on tissue or circulating tumor DNA (ctDNA) analysis and to confirm the feasibility of using ctDNA to identify HER2 expression for therapeutic targeting^[153].

Trastuzumab deruxtecan (T-DXd) is an antibody-drug conjugate formed by humanized anti-HER2 antibody with a topoisomerase I inhibitor payloads. Its effectiveness was demonstrated in the DESTINY-CRC01 phase II trial^[154]. Phase II DESTINY-CRC02 was designed to assess the efficacy of T-DXd in patients with HER2-amplified mCRC at a lower dose (5.4-mg/kg) compared to that in other studies. Preliminary data showed that T-DXd may be effective in RAS-MT mCRC, which is different from other trials of anti-HER2 regimens^[155].

Tucatinib, a selective oral small-molecule inhibitor of HER2, has shown promising efficacy in HER2-positive metastatic breast cancer^[156]. In xenograft models of patients with HER-positive CRC, tucatinib showed significant antitumor activity. When combined with trastuzumab, the effect of tumor growth inhibition becomes more pronounced^[156]. This provided the foundation for the MOUNTAINEER study, in which 26 patients with RAS-WT HER2 positive mCRC received tucatinib with trastuzumab. The results showed ORR of 55%, with impressive mPFS of 6.2 mo and OS of 17.3 mo respectively^[157,158].

MET amplification/activation and MET inhibitors: The phosphorylation of MET results in the activation of PI3K/AKT and RAS/RAF/MAPK to improve tumor cell growth^[159]. The dual blockade of MET and EGFR could provide a new therapeutic strategy for CRC patients with MET-driven acquired resistance to anti-EGFR. A phase II clinical study evaluated the efficacy of tivantinib and cetuximab in 41 patients with secondary resistance to anti-EGFR and MET overexpression. In the first stage, the study showed promising results with a DCR of 52.4%. However, the study did not reach the primary endpoint during the second stage, and only four patients achieved an objective response instead of five as expected. Whereas the survival results were still encouraging, with mPFS of 2.6 mo and mOS of 9.2 mo respectively^[160]. Crizotinib is another c-MET inhibitor that has been proven to increase sensitivity to radiotherapy in patients with cetuximab-resistant KRAS-MT mCRC^[161].

Rechallenge of anti- EGFR therapy

RAS mutations and EGFR ectodomain clones that appeared during anti-EGFR treatment would subside with antibody discontinuation, resulting in a decrease in the abundance of resistance mutations in the RAS/EGFR alleles, thereby restoring sensitivity to anti-EGFR regimens. However, not all patients would benefit from the rechallenge therapy. Utilizing ctDNA is crucial for the dynamic monitoring of acquired resistance and helps to guide treatment decisions, especially for EGFR rechallenge^[20]. Khan *et al*^[162] found that resistance mutations can be detected in the blood a few months before disease progression. The landmark observations were from the CRICKET and CAVE clinical trials. In the CRICKET single-arm phase II study, patients treated with first-line cetuximab plus irinotecan with at least six months of PFS were recruited and achieved an ORR of 21% and DCR of 54% to

anti-EGFR rechallenge, respectively. All patients with PR had baseline RAS-WT, and these patients experienced longer PFS than patients with ctDNA RAS-MT (4.0 mo vs 1.9 mo)^[163]. In CAVE phase II, single-arm clinical trial, combination of cetuximab and avelumab has been proved to be an effective rechallenge strategy in mCRC patients with RAS-WT, and mOS reached 17.3 mo in patients with RAS/BRAF-WT ctDNA prior to anti-EGFR rechallenge^[164]. The REMARRY and PURSUIT trials confirmed that plasma baseline RAS status could be a predictive indicator of rechallenge with anti-EGFR mAbs in patients with mCRC. In addition, this study demonstrated the effect of other gene mutations on the efficacy of rechallenge with anti-EGFR mAbs, including BRAF, EGFR, HER2, MET, and PIK3CA^[165].

OVERCOMING RESISTANCE TO ANTI-VEGF THERAPY

Vanucizumab targets both VEGF-A and angiopoietin-2, and has manageable safety and promising anticancer effects in a phase I study^[166]. Although compensatory activation of the c-MET pathway contributes to the loss of anti-VEGF drug efficacy, evidence for dual targeting of c-MET and VEGF in mCRC is rare^[47]. Famitinib is a novel TKI targeting VEGFR2, PDGFR β , and KIT^[167]. In a phase II trial from China, this drug correlated with an improvement in PFS (2.8 mo vs 1.5 mo) and DCR (59.8% vs 31.4%) compared to placebo for patients with chemo-refractory mCRC^[168]. In addition, the combination of bevacizumab and trifluridine/tipiracil is currently being tested in mCRC based on the promising results of preclinical and early-phase clinical trials^[168,169].

TARGETING CELL CYCLE

WEE1 is a significant part of the G2/M checkpoint through its actions on CDK1 to regulate the cell cycle^[170]. Adavosertib (AZD1775) is the first small-molecule inhibitor of WEE1 kinase, which has been tested in combination with chemotherapy and radiotherapy in several tumors. In addition, the protein p53 is also a pivotal factor in cell cycle arrest, inducing the inactivation of the G1/S phase checkpoint, thereby making tumor cells more dependent on the regulation of the G2/M checkpoint. Almost all tumor cells deactivate p53, most of which is mediated by TP53 mutations^[171]. A randomized trial (FOCUS4-C) evaluated adavosertib in patients with KRAS and TP53 MT mCRC. Patients with both mutations and stable or responding to chemotherapy were randomly assigned to two groups: Adavosertib and active monitoring (AM). Patients who received adavosertib showed an advantage in PFS compared to those on AM (3.61 mo vs 1.87 mo), although there was no significant difference in OS between the two groups (14 mo vs 18 mo)^[172]. In addition, the combination of adavosertib and irinotecan as second-line treatment for mCRC patients with KRAS or BRAF MT is being evaluated in a phase I trial (NCT02906059). Other WEE1 inhibitors have also been explored.

OTHER TARGETS: NEUROTROPHIC TROPOMYOSIN RECEPTOR KINASE, ANAPLASTIC LYMPHOMA KINASE, ROS1 FUSION

Gene fusions of neurotrophic tropomyosin receptor kinase (NTRK) genes have been found in 1.5% of all CRCs, and mainly in right-sided, MSI-H, and RAS/BRAF-WT tumors^[173,174]. Larotrectinib and entrectinib, which are highly selective inhibitors of TRKA, TRKB, and TRK, were approved by the FDA in November 2018 and August 2019, respectively^[175,176]. In a study by Doebele *et al*^[177],

larotrectinib achieved an ORR of 75%, and entrectinib produced 57%. The phase II NAVIGATE trial reported that larotrectinib generated an ORR of 50%, DCR of 100%, mPFS of 5.5 mo and mOS of 29.4 mo in 10 mCRC patients^[178]. Anaplastic lymphoma kinase (ALK), proto-oncogene 1, and receptor tyrosine kinase (ROS) are common driver genes in non-small cell lung cancer, but are extremely rare in mCRC, with an incidence of $\leq 1\%$ ^[179,180]. In preclinical studies, the combination of crizotinib and mitomycin C appeared to have synergistic effects in CRC^[181], and a series of trials on crizotinib are in progress.

IMMUNOTHERAPY

ICIs aim to improve immune surveillance and suppression by preventing tumor evasion from T-cell detection^[182]. Currently, ICIs have been investigated for the treatment of multiple solid tumors with promising outcomes. Pembrolizumab, a humanized IgG4 antibody, was the first PD-1 blocker to gain FDA approval for CRC treatment in 2017. Initial data from the randomized phase III KEYNOTE-177 study showed that pembrolizumab as first-line therapy *vs* standard chemotherapy improved PFS in patients with mismatch repair deficient (dMMR) or MSI-high (MSI-H) CRC^[183]. Recently, the study published the final results: Compared with chemotherapy, pembrolizumab did not provide a profound advantage in OS because the prespecified α of 0.025 was not achieved. However, in term of mPFS, the pembrolizumab group was superior to chemotherapy group (16.5 mo *vs* 8.2 mo)^[184]. Nivolumab, another humanized IgG4-based PD-1 antibody, approved by FDA for dMMR or MSI-H CRC in the same year on the basis of CheckMate-142 trial. The ORR reached 31.1%, regardless of the tumor PD-L1 expression, and 1-year PFS and OS rates were 50.4% and 73.4%, respectively^[185]. Combined nivolumab with CTLA-4 inhibitor ipilimumab outperforms single-agent immunotherapy

with acceptable adverse events. This combination regimen helped patients with dMMR or MSI-H mCRC achieve a PFS of 77% and an OS of 83% at 1 year, with an ORR of 60% and a DCR of 84%. Based on these data, the FDA has approved nivolumab and ipilimumab for patients with chemotherapy-refractory mCRC^[186].

Patients with MMR proficient (pMMR) or microsatellite stable (MSS) mCRC, which is also called “cold” tumor, could not obtain satisfactory results from ICIs; yet this subgroup accounts for the majority of CRC. Resistance mechanisms to immune checkpoint blockade in MSS mCRC include the loss of neoantigens, abnormal cell signaling, and immunosuppression^[187]. Currently, strategies to improve ICIs responses in patients with pMMR and MSS CRC, such as in combination with other approaches including chemotherapy, radiotherapy, targeted agents, and other immune checkpoint modulators are being explored^[182,188,189]. For these patients, the combination of PD-1/PD-L1 and CTLA-4-blocking antibodies may have manageable safety and empowering antitumor activity^[187]. In a clinical trial (NCT02754856), 21 patients with pMMR with liver-only metastases received durvalumab and tremelimumab prior to surgery. In the results, prolonged relapse free survival (RFS) as well as T cell activation was observed^[190]. Increasing evidence indicates that chemotherapy can boost the immune system by adjusting the immune microenvironment or directly stimulating antitumor responses^[191]. Dosset *et al* suggested that 5-FU plus oxaliplatin may be the most effective chemotherapy regimen for activating PD-L1 expression and CD8 recruitment. A phase I/II study explored the safety and efficacy of durvalumab plus tremelimumab combined with FOLFOX in mCRC patients with MSS and RAS mutated status^[192]. In a phase II trial, 43 patients with mCRC with MSS RAS/BRAF WT received chemotherapy, cetuximab, and avelumab. Among them, four patients achieved complete response; the ORR and DCR were 81% and 89%, respectively^[193]. Regorafenib plus

nivolumab also showed an inspiring 7.9-mo⁴ median PFS for MSS mCRC in the phase Ib trial REGONIVO (NCT03406871)^[194]. Radiotherapy can also promote T cell infiltration into cold tumors and has been used to improve immunotherapy efficacy^[195]. A phase II study enrolled 24⁴ patients with chemotherapy-refractory MSS mCRC who received durvalumab, tremelimumab, and radiotherapy²². The results showed that the median OS was 11.4 mo, and the median PFS was 1.8 mo^[196]. However, the optimal timing for the combination of immunotherapy and radiotherapy needs to be further explored. In addition, adoptive cellular therapy (ACT), as an emerging immunotherapy, has developed rapidly in several clinical studies and has been widely proven to have therapeutic efficacy in hematological malignancies. Carcinoembryonic antigen (CEA) is the most commonly researched target¹ of chimeric antigen receptor (CAR) T cells for the treatment of mCRC. Chengcheng *et al*²⁶ recruited 10 relapsed and refractory mCRC patients to evaluate the efficacy and safety of CAR-T therapy. Nearly 70% of patients achieved stable tumor control after treatment. In long-term observations, the serum CEA level decreased dramatically in most patients^[197]. Moreover, the key factor⁴ to transform “cold” tumors into “hot” tumors⁴ is to stimulate immune response, and cancer vaccine is an ideal strategy. Currently, several vaccines have been studied for CRC, including autologous, dendritic cells, viral vectors, and peptide-based vaccines^[198]. Relevant clinical trials⁴ are still in their early stages, and more research is needed to demonstrate its therapeutic potential^[199-201].

THE ROLE OF THE TUMOR MICROENVIRONMENT

Tumor microenvironment (TME) plasticity is a powerful driver of drug resistance. The TME contains not only the extracellular matrix (ECM), but also immune cells, endothelial cells, fibroblasts, and signaling molecules, including exosomes, chemokines, and

cytokines^[202]. The ECM is the main component that is remodeled during oncogenesis⁸ and progression. Collagen plays an important role in resisting tumor invasion and is regarded as a structural barrier. It was found that enriched collagen activates the PI3K/AKT signaling pathway via integrin $\alpha 2\beta 1$ to sustain tumor growth. Notably, the inhibitor of integrin $\alpha 2\beta 1$ can prevent metastasis, and the combination of integrin $\alpha 2\beta 1$ inhibitor with chemotherapy showed improved curative effects^[203]. In addition, the expression of type I collagen⁶⁸, matrix metalloproteinase (MMP-2), and MMP-9 upregulated in primary CRC, which participated in the degradation and regeneration of the ECM^[204]. These findings will provide new ideas for the future treatment of mCRC. Fibroblasts are mesenchymal cells that secrete mitogenic growth factors such as FGF1, FGF2, hepatocyte growth factor (HGF), transforming growth factor beta-1 (TGF- $\beta 1$), and TGF- $\beta 2$ ^[205]. Luraghi *et al*^[206] found that HGF binds to MET receptors and stimulates MAPK and AKT to induce cetuximab resistance. This provides a theoretical basis for the concomitant blockade of FGFR and EGFR to reverse resistance to anti-EGFR mAbs. The combination of BLU9931 (an FGFR4 inhibitor) and cetuximab showed improved antitumor activity compared to cetuximab alone^[207]. Exosomes exhibit potential antigenicity and can induce powerful immune responses^[208]. Several studies have indicated that exosomes can be used as vaccines for mCRC. A phase I clinical trial¹⁸ has shown that the combination of ascites-derived exosomes (Aex) and granulocyte-macrophage colony-stimulating factor (GM-CSF) is a feasible alternative choice in the immunotherapy of mCRC^[200].

WNT SIGNALING

The Wnt gene is a member of the coiled family of transmembrane receptors consisting of multiple glycoproteins and is a co-receptor for the lipoprotein receptor-related protein (LRP) family and other downstream signaling proteins^[209,210]. Wnt/ β -catenin signaling is the most classical pathway in Wnt signaling, and its key steps are protein stabilization and nuclear translocation. When Wnt ligands are not activated, a complex formed by adenomatous polyposis coli (APC) protein, framework protein Axin, glycogen synthase kinase 3 β (GSK3 β), and casein kinase 1 (CK1) will degrade β -catenin^[211,212]. In the case of Wnt activation, Wnt ligands integrate with receptors, resulting in the phosphorylation of LRP by CK1 and GSK3, and recruit proteins such as axisprotein (Axin) and dishevelled to the cell membrane, thereby disrupting the formation of the complexes that could degrade β -catenin^[213,214]. Undegraded β -catenin accumulates in the cytoplasm, and when it reaches a sufficient concentration, free β -catenin is transferred to the nucleus and binds to the transcription factor/lymphocyte-enhancing factor (TCF/LEF), inducing cell proliferation, differentiation, and maturation^[214,215]. Normal transduction of Wnt signaling facilitates the regulation of intestinal function^[216,217]. Abnormal activation of Wnt/ β -catenin signaling is found in more than 80% of CRCs, which leads to the accumulation of nuclear β -catenin and is associated with a poor prognosis^[218]. Studies have shown that Wnt/ β -catenin signaling mediates CRC resistance via three pathways: Tumor stem cells, non-coding RNAs (ncRNAs), and disordered TME^[219-222].

β -catenin is a crucial target of Wnt signaling, thus preventing the protein-protein connection between TCF and β -catenin to suppress tumor cell growth^[223]. The earliest studies utilized high-throughput enzyme-linked immunosorbent assay (ELISA) to detect the β -catenin-TCF connection, and eight compounds were initially screened, of which PKF115-584 and CGP049090 proved to be the most potent material^[223]. Moreover, PKF115-584 and CGP049090 also interfere with β -catenin-APC interaction^[224-226].

Another study found that ⁷2,4-diamino-quinazoline (Table 1) exhibited solubility, metabolic stability, and oral bioavailability by preventing the β -catenin-TCF4 pathway^[227,228]. Porcupine²⁸ is a member of the membrane-bound O-acyltransferase (MBOAT) family. It adds a palmitoyl group to the protein, which is involved in Wnt signaling^[229]. Small molecule compounds such as IWP, C59, and ETC-159 can target porcupine, thereby inhibiting the activity of the Wnt signaling pathways^[230]. In addition, many natural compounds, such as curcumin, soybean-derived isoflavone-phytoestrogen genistein, and berberine, inhibit the Wnt signaling pathway by acting on different targets^[231,232]. These natural compounds have the potential to treat CRC and deserve further investigation.

CONCLUSION

The heterogeneity and molecular diversity of CRC promote drug resistance and are associated with adverse outcomes. The field of CRC has continued to develop over the last few decades, leading to a better understanding of its occurrence and development. This review summarizes the currently available targeted therapies, drug resistance mechanisms, and potential treatments for mCRC. It is not difficult to find that a wider range of molecular markers is needed to assess the efficacy of targeted therapy and obtain optimal outcomes for patients. Excitingly, high-sensitivity genome sequencing methods and the application of liquid biopsy¹³ have provided deeper insight into the molecular evolution and mechanisms of resistance to treatment. In addition to the targets mentioned in this review, there are other new targets that correlate with the prognosis of patients with mCRC. The aim is to

develop more specific drugs for these targets to adopt more individualized treatments and eventually improve the prognosis of patients with mCRC.

1
Table 1 Strategies to reverse targeted therapy resistance in clinical trials

Targets	Agents	Phase	Condition	Treatment	Main outcomes
1 EGFR ECD	Sym004	Phase I	KRAS WT mCRC	Sym004	mOS: 12.8 mo
1 EGFR S468R	Necitumumab	Phase II	First-line mCRC	Necitumumab mFOLFOX6	+ ORR: 63.6%
KRAS ^{G12C}	Sotorasib (AMG 510)	Phase I	Solid tumors	Sotorasib	ORR: 7.1%; DCR: 73.8%
KRAS ^{G12C}	Adagrasib (MRTX849)	Phase II	Solid tumors	Adagrasib	ORR: 17%; DCR: 94%
BRAF	Vemurafenib	Pilot trial	Previously treated	Vemurafenib panitumumab	+ DCR: 83%
BRAF	Vemurafenib	Phase I	Previously treated	Irinotecan + Vemurafenib	ORR: 35%; DCR: 88%; mPFS: 7.7m
BRAF	Vemurafenib	SWOG-S1406; phase II	Previously treated	Irinotecan + Cetuximab + Vemurafenib	mPFS: 4.2 mo; ORR: 17%; DCR:

					65%
BRAF	Encorafenib	BEACON; phase III	Previously treated	Encorafenib + binimetinib + cetuximab	mOS: 9.3; ORR: 26.8%
BRAF	Dabrafenib	Phase I/II	BRAF WT	Dabrafenib + trametinib	ORR: 12%
HER2	Dual-targeted drugs	HERACLE A; phase II	KRAS WT	Trastuzumab + lapatinib	mOS: 11.5 mo; mPFS: 5.0 mo; ORR: 30%
HER2	Pertuzumab; DM1	T- HERACLE B; phase II	KRAS/BRAF WT	Pertuzumab + trastuzumab	ORR: 9.7%; mPFS: 4.1 mo
HER2	Trastuzumab pertuzumab	MyPathway; phase II	Previously treated	Trastuzumab + pertuzumab	ORR: 32%; mOS: 11.5 mo; mPFS: 2.9 mo
HER2	Trastuzumab pertuzumab	TRIUMPH phase II	KRAS WT	Trastuzumab + pertuzumab	ORR: 30%; mOS: 10.1 mo; mPFS: 4.0 mo
HER2	ADC	DESTINY- CRC01phase II	Previously treated	Trastuzumab deruxtecan (T-DXd)	ORR: 45.3%; mOS: 15.5 mo; mPFS: 6.9 mo

HER2	tucatinib	MOUNTAINEER; phase II	Previously treated	Tucatinib + trastuzumab	ORR: 52%; mOS: 18.7 mo; mPFS: 8.1 mo
MET	Tivantinib	Phase II	KRAS WT; previously treated	Tivantinib + irinotecan + cetuximab	mPFS: 8.3 mo; ORR: 44%; mOS: 19.8 mo
EGFR rechallenge	Irinotecan	CRICKET; phase II	KRAS/BRAF WT; third-line	Irinotecan + cetuximab	ORR: 21%; DCR: 54%; mOS: 9.8 mo; mPFS: 3.4 mo
EGFR rechallenge	Avelumab	CAVE; phase II	KRAS WT; third-line	Avelumab + cetuximab	mOS: 11.6 mo; mPFS: 3.6 mo; ORR: 7.8%; DCR: 65%
EGFR rechallenge	Irinotecan	REMARRY and PURSUIT; phase II	KRAS/BRAF WT	Panitumumab + irinotecan	ORR: 14%; mPFS: 3.6 mo
VEGF	Vanucizumab	Phase I	Solid tumors	Vanucizumab	DCR: 59.8%; mPFS: 2.8 mo
VEGF	Bevacizumab	TRIBE; phase II	mCRC	Bevacizumab +	mPFS: 12.1 mo;

				FOLFOXIRI	mOS: 30.9 mo
VEGF	Aflibercept	VALOUR; phase III	Previously treated	Aflibercept + FOLFIRI	mOS: 13.5 mo; mPFS: 6.9 mo
VEGF	Aflibercept	AFFIRM; phase II	First-line	Aflibercept + FOLFOX	mPFS: 8.48 mo; 12mPFS: 25.8%
VEGF	Ramucirumab	RAISE; phase III	Second-line	Ramucirumab + FOLFIRI	mOS: 13.3 mo
VEGF	Bevacizumab	C-TASK FORCE; phase I/II	Previously treated	Bevacizumab + trifluridine/tipiracil	16 wk PFS: 46.9%
TKI	Famitinib	Phase II	Previously treated	Famitinib	mPFS: 2.8 mo; mOS: 7.4 mo; DCR: 59.8%; ORR: 2.2%
NTRK	Larotrectinib	NAVIGATE; phase I/II	Solid tumors	Larotrectinib	ORR: 50%; DCR: 100%; mPFS: 5.5 mo; mOS: 29.4 mo
³⁴ PD-1/PD-L1	Pembrolizumab	KEYNOTE-177; phase III	dMMR/MSI-H CRC; first-line	Pembrolizumab	mPFS: 16.5 mo; mOS: NA
PD-1/PD-L1	Nivolumab	CheckMate-142;	dMMR/MSI-H CRC;	Nivolumab	ORR: 60%; DCR:

		phase II	previously treated		84%
PD-1/PD-L1	Durvalumab	Phase I	Solid tumors	Durvalumab + Tremelimumab	DCR: 36.4%
PD-1/PD-L1	Durvalumab	Phase Ib/II	MSS RAS/BRAF WT	Durvalumab + Tremelimumab	ORR: 81%; DCR: 89%
				FOLFOX	
³⁴ PD-1/PD-L1	Nivolumab	Phase Ib	MSS	Nivolumab + Regorafenib	mPFS: 7.9 mo
PD-1/PD-L1	Durvalumab	Phase II	Chemotherapy-refractory MSS	Durvalumab + Tremelimumab	ORR: 8.3%; mPFS: 1.8 mo; mOS: 11.4 mo
				Radiotherapy	

² mCRC: Metastatic colorectal cancer; EGFR: Epidermal growth factor receptor; VEGF: Vascular endothelial growth factor; TKI: Tyrosine kinase inhibitors; ¹¹ ADC: Antibody-drug conjugate; ORR: Overall response rate; ³¹ mOS: Median overall survival; mPFS: Median progression-free survival; DCR: Disease control rate; PD-1: Programmed cell death protein 1; ⁵² PD-L1: Programmed death-ligand 1; MSS: Microsatellite stable; FOLFOX: Leucovorin, 5-fluorouracil, and oxaliplatin; ⁷⁵ FOLFIRI: Leucovorin, 5-fluorouracil, and irinotecan; T-DXd: Trastuzumab deruxtecan; ⁷⁶ HER: Human epidermal growth factor receptor.

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