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**Resistance to targeted therapy in metastatic colorectal cancer: Current status and new developments**

Tang YL *et al*. Targeted therapy resistance in mCRC

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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) 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 to targeted therapy, along with searching for novel effective regimens, is a constant challenge in the mCRC treatment, and it is also a hot research topic. In this review, we focus on the current status on resistance to existing targeted therapies in mCRC and discuss future developments.

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

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**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 the early stage of the 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) and 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 (mAbs), 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 CRC; 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 (WT) 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 a 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**

***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 WT RAS mutation[13,15]. Patients with BRAF mutations failed to respond to anti-EGFR therapy and BRAF mutations were independent of RAS mutations. Therefore, the European Society of Medical Oncology (ESMO) and National Comprehensive Cancer Network (NCCN) guidelines recommend anti-EGFR therapy 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 Oncology recommends that populations with left-sided tumors *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 pathways are the main causes of resistance to anti-EGFR therapy, including RAS/RAF/MEK and PI3K/AKT/mTOR[31-33]. In addition, the activation 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].

***VEGF pathway***

Angiogenesis is essential for tumor initiation, development, and metastasis. Overexpression of the VEGF ligand family and their 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-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 that 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 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]. Thus, the development of new mAbs that can target different EGFR epitopes promises to reverse anti-EGFR resistance. Among different EGFR somatic sequence changes located in the extracellular domain (ECD), S492R affects cetuximab binding, but does not interrupt panitumumab binding, suggesting a rechallenge with panitumumab in patients resistant to cetuximab and developing an S492R mutation[50]. MM-151, an mAb that engages multiple epitopes on EGFR, interferes with EGFR signaling 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]. A multicenter, phase II clinical trial explored the 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, and best supportive care), subgroup analysis demonstrated that patients without EGFR ECD mutations had benefited in OS with Sym004 therapy[54]. Necitumumab is another approved EGFR antibody that can integrate with S468R, the most common cetuximab resistance substitution[55]. Necitumumab 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 demonstrated that not all KRAS-mutated patients develop resistance to anti-EGFR treatment[60]. It is unclear whether patients with KRASG13D gene mutations 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 precise because of 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, KRASG12C 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 KRASG12C. The anticancer activity of sotorasib in patients harboring the KRASG12C mutation has been demonstrated in a phase I trial. In the subgroup with colorectal cancer, 3 of 42 (7.1%) patients achieved partial response (PR), 28 (66.7%) experienced stable disease (SD), and the mPFS was 4.0 mo[67,68]. Adagrasib (MRTX849) is another covalent KRASG12C inhibitor that achieves an overall response rate (ORR) and disease control rate (DCR) of 17% (3/18) and 94% (17/18), respectively, in patients with KRASG12C CRC[69]. In addition, a phase III trial assessed the effect of adagrasib plus cetuximab as second-line treatment for KRASG12C MT mCRC[70]. Regrettably, KRASG12C is found in only 4% of CRCs, and KRASG12D 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 KRASG12D is much more difficult than that targeting KRASG12C because KRASG12D lacks cysteine, which can covalently bind to small molecular drugs. MRTX1133 is the first noncovalent and specific inhibitor of KRASG12D with preclinical data. The binding activity and selectivity of MRTX1133 were improved by optimizing the positions 2, 4, and 7 of pyrido[4,3-d] pyrimidine based on adagrasib. In the mouse models of xenograft tumors, MRTX1133 has been shown to inhibit KRAS signaling and exhibit robust antitumor activity[72]. In addition, Revolution Medicines have developed a covalent KRASG12D inhibitor that targets the activated state of KRAS. RM-036 forms a tri-complex with chaperone cyclophilin A and the on-state of KRASG12D and can selectively bind to aspartate of KRASG12D mutants, effectively inhibiting signal transduction. Treatment with RM-036 resulted in dramatic tumor regression and a complete response in several KRASG12D mutant xenograft models[73]. Overall, targeted therapies for KRASG12D 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 CRC models with KRASG12D and KRASG13D 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 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 *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 *in vivo* trials[86]. The efficacy of palbociclib and another CDK4/6 inhibitor, trametinib, was evaluated in a phase Ib study (NCT02065063). In a case report, 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 programmed cell death protein 1 (PD-1) and decrease the expression of programmed death-ligand 1 (PD-L1) in CRC with microsatellite instability (MSI)[88]. In preclinical models, sotorasib plus checkpoint inhibitor therapy (immune checkpoint inhibitors, ICIs) increased T cell infiltration and activation, thereby forming a tumor microenvironment (TME) 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, 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 exhibited induced energy crisis and cell death when exposed to high doses of vitamin C (AA)[99]. The favorable safety and preliminary efficacy of AA plus mFOLFOX6/FOLFIRI (irinotecan, 5-fluoruracil, and leucovorin) in mCRC have been proven in a phase I study. Relevant phase II and III studies are ongoing[100].

**Targeting BRAF mutant CRC:** BRAF mutations occur in approximately 12% of patients with CRC, and more than 95% of these mutations manifest as V600E substitution[101-104]. 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, most of which are mucinous adenocarcinomas. Moreover, BRAF-MT CRC mainly develops peritoneal and lymph node metastasis[105-107]. In addition, more than 50% of BRAF V600E mutant CRCs have a high MSI (MSI-H) status[108,109]. CRCs harboring these features are frequently sporadic and exhibit extensive DNA methylation of CpG islands[110,111]. 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. Sixteen patients received FOLFOXIRI plus bevacizumab, and 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]. The efficacy of vemurafenib combined with panitumumab in 15 patients with chemo-resistant BRAF-MT mCRC. The results of this trial included ten cases of tumor regression and two cases of stable disease 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. 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 microsatellite stable (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 into three groups: Encorafenib, binimetinib, and cetuximab; encorafenib and cetuximab; and irinotecan plus FOLFIRI or cetuximab (control group). The triplet therapy significantly improved the OS, with an mOS of 9.0 mo. The objective response rates 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 in 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] tested dabrafenib plus trametinib in 43 patients with BRAF V600-mutanted CRC, of whom five (12%) achieved partial response, and 24 (56%) achieved stable disease[136].

**PI3K/AKT activation and PI3K/AKT inhibitors:** PIK3CA encodes the p110α catalytic subunit of phosphatidylinositol 3-kinase (PI3K), found in 10%-20% of CRC patients[31]. PI3KA mutation and abnormal AKT/mTOR activation lead 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, 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 *et al*[141] found that HER2 positive patients were sensitive to dual regimens of trastuzumab and lapatinib but not to either agent alone. HERACLESA, a multicenter phase II trial, evaluated the efficacy of a combination of trastuzumab and lapatinib in mCRC patients with KRAS-WT and HER2 overexpression refractory to standard treatment[148]. HER2 positivity was defined by immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH): Tumors with a score of IHC 3 + in more than half of the cells, or with an 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: An mPFS of 5.0 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]. In the HERACLES-B study, a single-arm phase II trial, patients were treated with pertuzumab and trastuzumab (T-DM1). A total of 31 patients were evaluable, of whom 48% had more than four lines of previous therapies. 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 payload. 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 an ORR of 55%, with an 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 acquired MET-driven 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 an 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 6 mo 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, a 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 baseline plasma 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-VEGR 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, PDGFRβ, 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 who were 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, AND ROS1 FUSION**

Fusions of neurotrophic tropomyosin receptor kinase (NTRK) genes have been found in 1.5% of all CRCs, 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 Cocco *et al*[177], larotrectinib achieved an ORR of 75%, and entrectinib resulted in an ORR of 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 ten 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 ≤ 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-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, was 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]. Nivolumab combined with the 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 OS of 83% at 1 year, with an ORR of 60% and 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 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, and 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]. It was 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-mo 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 24 patients with chemotherapy-refractory MSS mCRC who received durvalumab, tremelimumab, and radiotherapy. 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 target of chimeric antigen receptor (CAR) T cells for the treatment of mCRC. Zhang *et al*[197] recruited ten 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 factor to transform “cold” tumors into “hot” tumors is to stimulate immune response, and cancer vaccine is an ideal strategy. Currently, several vaccines have been studied for CRC, including autologous vaccines, dendritic cells, viral vectors, and peptide-based vaccines[198]. Relevant clinical trials are still in their early stages, and more research is needed to demonstrate their therapeutic potential[199-201].

**ROLE OF 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 oncogenesis 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 α2β1 to sustain tumor growth. Notably, the inhibitor of integrin α2β1 can prevent metastasis, and the combination of integrin α2β1 inhibitor with chemotherapy showed improved curative effects[203]. In addition, the expression of type I collagen, matrix metalloproteinase (MMP-2), and MMP-9 is 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-β1), and TGF-β2[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 trial 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 protein 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 3β (GSK3β), 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 suppresses 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 2,4-diamino-quinazoline (Table 1) exhibited solubility, metabolic stability, and oral bioavailability by preventing the β-catenin-TCF4 pathway[227,228]. Porcupine 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 biopsy 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.

**REFERENCES**

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Miller KD**, Nogueira L, Mariotto AB, Rowland JH, Yabroff KR, Alfano CM, Jemal A, Kramer JL, Siegel RL. Cancer treatment and survivorship statistics, 2019. *CA Cancer J Clin* 2019; **69**: 363-385 [PMID: 31184787 DOI: 10.3322/caac.21565]

3 **Siegel RL**, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 177-193 [PMID: 28248415 DOI: 10.3322/caac.21395]

4 **Falcone A**, Ricci S, Brunetti I, Pfanner E, Allegrini G, Barbara C, Crinò L, Benedetti G, Evangelista W, Fanchini L, Cortesi E, Picone V, Vitello S, Chiara S, Granetto C, Porcile G, Fioretto L, Orlandini C, Andreuccetti M, Masi G; Gruppo Oncologico Nord Ovest. Phase III trial of infusional fluorouracil, leucovorin, oxaliplatin, and irinotecan (FOLFOXIRI) compared with infusional fluorouracil, leucovorin, and irinotecan (FOLFIRI) as first-line treatment for metastatic colorectal cancer: the Gruppo Oncologico Nord Ovest. *J Clin Oncol* 2007; **25**: 1670-1676 [PMID: 17470860 DOI: 10.1200/JCO.2006.09.0928]

5 **Fuchs CS**, Marshall J, Mitchell E, Wierzbicki R, Ganju V, Jeffery M, Schulz J, Richards D, Soufi-Mahjoubi R, Wang B, Barrueco J. Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. *J Clin Oncol* 2007; **25**: 4779-4786 [PMID: 17947725 DOI: 10.1200/JCO.2007.11.3357]

6 **Tournigand C**, André T, Achille E, Lledo G, Flesh M, Mery-Mignard D, Quinaux E, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C, de Gramont A. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2004; **22**: 229-237 [PMID: 14657227 DOI: 10.1200/JCO.2004.05.113]

7 **Cremolini C**, Loupakis F, Antoniotti C, Lupi C, Sensi E, Lonardi S, Mezi S, Tomasello G, Ronzoni M, Zaniboni A, Tonini G, Carlomagno C, Allegrini G, Chiara S, D'Amico M, Granetto C, Cazzaniga M, Boni L, Fontanini G, Falcone A. FOLFOXIRI plus bevacizumab versus FOLFIRI plus bevacizumab as first-line treatment of patients with metastatic colorectal cancer: updated overall survival and molecular subgroup analyses of the open-label, phase 3 TRIBE study. *Lancet Oncol* 2015; **16**: 1306-1315 [PMID: 26338525 DOI: 10.1016/S1470-2045(15)00122-9]

8 **Carlson RW**, Larsen JK, McClure J, Fitzgerald CL, Venook AP, Benson AB 3rd, Anderson BO. International adaptations of NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw* 2014; **12**: 643-648 [PMID: 24812133 DOI: 10.6004/jnccn.2021.0012]

9 **Personeni N**, Smiroldo V, Giunta EF, Prete MG, Rimassa L, Bregni G, Sclafani F. Tackling Refractory Metastatic Colorectal Cancer: Future Perspectives. *Cancers (Basel)* 2021; **13** [PMID: 34572729 DOI: 10.3390/cancers13184506]

10 **Van Cutsem E**, Cervantes A, Nordlinger B, Arnold D; ESMO Guidelines Working Group. Metastatic colorectal cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2014; **25 Suppl 3**: iii1-iii9 [PMID: 25190710 DOI: 10.1093/annonc/mdu260]

11 **Vera R**, Alonso V, Gállego J, González E, Guillén-Ponce C, Pericay C, Rivera F, Safont MJ, Valladares-Ayerbes M. Current controversies in the management of metastatic colorectal cancer. *Cancer Chemother Pharmacol* 2015; **76**: 659-677 [PMID: 26113053 DOI: 10.1007/s00280-015-2808-6]

12 **Cunningham D**, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, Van Cutsem E. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 2004; **351**: 337-345 [PMID: 15269313 DOI: 10.1056/NEJMoa033025]

13 **Van Cutsem E**, Köhne CH, Hitre E, Zaluski J, Chang Chien CR, Makhson A, D'Haens G, Pintér T, Lim R, Bodoky G, Roh JK, Folprecht G, Ruff P, Stroh C, Tejpar S, Schlichting M, Nippgen J, Rougier P. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; **360**: 1408-1417 [PMID: 19339720 DOI: 10.1056/NEJMoa0805019]

14 **Hurwitz H**, Fehrenbacher L, Novotny W. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2019; **350**: 2335-2342 [DOI: 10.1016/s0305-7372(04)00149-5]

15 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Tian Y, Xu F, Sidhu R. Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. *Ann Oncol* 2014; **25**: 1346-1355 [PMID: 24718886 DOI: 10.1093/annonc/mdu141]

16 **Yarom N**, Jonker DJ. The role of the epidermal growth factor receptor in the mechanism and treatment of colorectal cancer. *Discov Med* 2011; **11**: 95-105 [PMID: 21356164]

17 **Douillard JY**, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Błasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M, Gansert J. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol* 2010; **28**: 4697-4705 [PMID: 20921465 DOI: 10.1200/JCO.2009.27.4860]

18 **Hurwitz HI**, Tebbutt NC, Kabbinavar F, Giantonio BJ, Guan ZZ, Mitchell L, Waterkamp D, Tabernero J. Efficacy and safety of bevacizumab in metastatic colorectal cancer: pooled analysis from seven randomized controlled trials. *Oncologist* 2013; **18**: 1004-1012 [PMID: 23881988 DOI: 10.1634/theoncologist.2013-0107]

19 **Goldberg RM**, Sargent DJ, Morton RF, Fuchs CS, Ramanathan RK, Williamson SK, Findlay BP, Pitot HC, Alberts SR. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2004; **22**: 23-30 [PMID: 14665611 DOI: 10.1200/JCO.2004.09.046]

20 **Venook AP**, Niedzwiecki D, Lenz HJ, Innocenti F, Fruth B, Meyerhardt JA, Schrag D, Greene C, O'Neil BH, Atkins JN, Berry S, Polite BN, O'Reilly EM, Goldberg RM, Hochster HS, Schilsky RL, Bertagnolli MM, El-Khoueiry AB, Watson P, Benson AB 3rd, Mulkerin DL, Mayer RJ, Blanke C. Effect of First-Line Chemotherapy Combined With Cetuximab or Bevacizumab on Overall Survival in Patients With KRAS Wild-Type Advanced or Metastatic Colorectal Cancer: A Randomized Clinical Trial. *JAMA* 2017; **317**: 2392-2401 [PMID: 28632865 DOI: 10.1001/jama.2017.7105]

21 **Schwartzberg LS**, Rivera F, Karthaus M, Fasola G, Canon JL, Hecht JR, Yu H, Oliner KS, Go WY. PEAK: a randomized, multicenter phase II study of panitumumab plus modified fluorouracil, leucovorin, and oxaliplatin (mFOLFOX6) or bevacizumab plus mFOLFOX6 in patients with previously untreated, unresectable, wild-type KRAS exon 2 metastatic colorectal cancer. *J Clin Oncol* 2014; **32**: 2240-2247 [PMID: 24687833 DOI: 10.1200/JCO.2013.53.2473]

22 **Van Cutsem E**, Tabernero J, Lakomy R, Prenen H, Prausová J, Macarulla T, Ruff P, van Hazel GA, Moiseyenko V, Ferry D, McKendrick J, Polikoff J, Tellier A, Castan R, Allegra C. Addition of aflibercept 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**: 3499-3506 [PMID: 22949147 DOI: 10.1200/JCO.2012.42.8201]

23 **Shitara K**, Yamanaka T, Denda T, Tsuji Y, Shinozaki K, Komatsu Y, Kobayashi Y, Furuse J, Okuda H, Asayama M, Akiyoshi K, Kagawa Y, Kato T, Oki E, Ando T, Hagiwara Y, Ohashi Y, Yoshino T. REVERCE: a randomized phase II study of regorafenib followed by cetuximab versus the reverse sequence for previously treated metastatic colorectal cancer patients. *Ann Oncol* 2019; **30**: 259-265 [PMID: 30508156 DOI: 10.1093/annonc/mdy526]

24 **Tabernero J**, Yoshino T, Cohn AL, Obermannova R, Bodoky G, Garcia-Carbonero R, Ciuleanu TE, Portnoy DC, Van Cutsem E, Grothey A, Prausová J, Garcia-Alfonso P, Yamazaki K, Clingan PR, Lonardi S, Kim TW, Simms L, Chang SC, Nasroulah F; RAISE Study Investigators. Ramucirumab versus placebo in combination with second-line FOLFIRI in patients with metastatic colorectal carcinoma that progressed during or after first-line therapy with bevacizumab, oxaliplatin, and a fluoropyrimidine (RAISE): a randomised, double-blind, multicentre, phase 3 study. *Lancet Oncol* 2015; **16**: 499-508 [PMID: 25877855 DOI: 10.1016/S1470-2045(15)70127-0]

25 **Grothey A**, Van Cutsem E, Sobrero A, Siena S, Falcone A, Ychou M, Humblet Y, Bouché O, Mineur L, Barone C, Adenis A, Tabernero J, Yoshino T, Lenz HJ, Goldberg RM, Sargent DJ, Cihon F, Cupit L, Wagner A, Laurent D; CORRECT Study Group. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet* 2013; **381**: 303-312 [PMID: 23177514 DOI: 10.1016/S0140-6736(12)61900-X]

26 **Li J**, Qin S, Xu RH, Shen L, Xu J, Bai Y, Yang L, Deng Y, Chen ZD, Zhong H, Pan H, Guo W, Shu Y, Yuan Y, Zhou J, Xu N, Liu T, Ma D, Wu C, Cheng Y, Chen D, Li W, Sun S, Yu Z, Cao P, Chen H, Wang J, Wang S, Wang H, Fan S, Hua Y, Su W. Effect of Fruquintinib vs Placebo on Overall Survival in Patients With Previously Treated Metastatic Colorectal Cancer: The FRESCO Randomized Clinical Trial. *JAMA* 2018; **319**: 2486-2496 [PMID: 29946728 DOI: 10.1001/jama.2018.7855]

27 **Roskoski R Jr**. The ErbB/HER family of protein-tyrosine kinases and cancer. *Pharmacol Res* 2014; **79**: 34-74 [PMID: 24269963 DOI: 10.1016/j.phrs.2013.11.002]

28 **Anderson VM**, Fisniku LK, Khaleeli Z, Summers MM, Penny SA, Altmann DR, Thompson AJ, Ron MA, Miller DH. Hippocampal atrophy in relapsing-remitting and primary progressive MS: a comparative study. *Mult Scler* 2010; **16**: 1083-1090 [PMID: 20630904 DOI: 10.1177/1352458510374893]

29 **Dzunic M**, Petkovic I, Cvetanovic A. Current and future targets and therapies for metastatic colorectal cancer. *J Buon* 2019; **24**: 1785-1792 [DOI: 10.37155/2661-4766-0102-4]

30 **Dzunic M**, Petkovic I, Cvetanovic A, Vrbic S, Pejcic I. Current and future targets and therapies in metastatic colorectal cancer. *J BUON* 2019; **24**: 1785-1792 [PMID: 31786838 DOI: 10.1200/JCO.2007.13.2183]

31 **De Roock W**, Claes B, Bernasconi D, De Schutter J, Biesmans B, Fountzilas G, Kalogeras KT, Kotoula V, Papamichael D, Laurent-Puig P, Penault-Llorca F, Rougier P, Vincenzi B, Santini D, Tonini G, Cappuzzo F, Frattini M, Molinari F, Saletti P, De Dosso S, Martini M, Bardelli A, Siena S, Sartore-Bianchi A, Tabernero J, Macarulla T, Di Fiore F, Gangloff AO, Ciardiello F, Pfeiffer P, Qvortrup C, Hansen TP, Van Cutsem E, Piessevaux H, Lambrechts D, Delorenzi M, Tejpar S. Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis. *Lancet Oncol* 2010; **11**: 753-762 [PMID: 20619739 DOI: 10.1016/S1470-2045(10)70130-3]

32 **Bertotti A**, Papp E, Jones S, Adleff V, Anagnostou V, Lupo B, Sausen M, Phallen J, Hruban CA, Tokheim C, Niknafs N, Nesselbush M, Lytle K, Sassi F, Cottino F, Migliardi G, Zanella ER, Ribero D, Russolillo N, Mellano A, Muratore A, Paraluppi G, Salizzoni M, Marsoni S, Kragh M, Lantto J, Cassingena A, Li QK, Karchin R, Scharpf R, Sartore-Bianchi A, Siena S, Diaz LA Jr, Trusolino L, Velculescu VE. The genomic landscape of response to EGFR blockade in colorectal cancer. *Nature* 2015; **526**: 263-267 [PMID: 26416732 DOI: 10.1038/nature14969]

33 **Karapetis CS**, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, Simes RJ, Chalchal H, Shapiro JD, Robitaille S, Price TJ, Shepherd L, Au HJ, Langer C, Moore MJ, Zalcberg JR. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med* 2008; **359**: 1757-1765 [PMID: 18946061 DOI: 10.1056/NEJMoa0804385]

34 **Bardelli A**, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, Sartore-Bianchi A, Scala E, Cassingena A, Zecchin D, Apicella M, Migliardi G, Galimi F, Lauricella C, Zanon C, Perera T, Veronese S, Corti G, Amatu A, Gambacorta M, Diaz LA Jr, Sausen M, Velculescu VE, Comoglio P, Trusolino L, Di Nicolantonio F, Giordano S, Siena S. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. *Cancer Discov* 2013; **3**: 658-673 [PMID: 23729478 DOI: 10.1158/2159-8290.CD-12-0558]

35 **Huang F**, Xu LA, Khambata-Ford S. Correlation between gene expression of IGF-1R pathway markers and cetuximab benefit in metastatic colorectal cancer. *Clin Cancer Res* 2012; **18**: 1156-1166 [PMID: 22294722 DOI: 10.1158/1078-0432.CCR-11-1135]

36 **Kavuri SM**, Jain N, Galimi F, Cottino F, Leto SM, Migliardi G, Searleman AC, Shen W, Monsey J, Trusolino L, Jacobs SA, Bertotti A, Bose R. HER2 activating mutations are targets for colorectal cancer treatment. *Cancer Discov* 2015; **5**: 832-841 [PMID: 26243863 DOI: 10.1158/2159-8290.CD-14-1211]

37 **Sidaway P**. Microenvironment plasticity confers cetuximab resistance. *Nat Rev Clin Oncol* 2019; **16**: 527 [PMID: 31324873 DOI: 10.1038/s41571-019-0259-4]

38 **Ferrara N**, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003; **9**: 669-676 [PMID: 12778165 DOI: 10.1038/nm0603-669]

39 **Heinemann V**, von Weikersthal LF, Decker T, Kiani A, Vehling-Kaiser U, Al-Batran SE, Heintges T, Lerchenmüller C, Kahl C, Seipelt G, Kullmann F, Stauch M, Scheithauer W, Hielscher J, Scholz M, Müller S, Link H, Niederle N, Rost A, Höffkes HG, Moehler M, Lindig RU, Modest DP, Rossius L, Kirchner T, Jung A, Stintzing S. FOLFIRI plus cetuximab versus FOLFIRI plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer (FIRE-3): a randomised, open-label, phase 3 trial. *Lancet Oncol* 2014; **15**: 1065-1075 [PMID: 25088940 DOI: 10.1016/S1470-2045（14）70330-4]

40 **Kopetz S**, Hoff PM, Morris JS, Wolff RA, Eng C, Glover KY, Adinin R, Overman MJ, Valero V, Wen S, Lieu C, Yan S, Tran HT, Ellis LM, Abbruzzese JL, Heymach JV. Phase II trial of infusional fluorouracil, irinotecan, and bevacizumab for metastatic colorectal cancer: efficacy and circulating angiogenic biomarkers associated with therapeutic resistance. *J Clin Oncol* 2010; **28**: 453-459 [PMID: 20008624 DOI: 10.1200/JCO.2009.24.8252]

41 **Chiron M**, Bagley RG, Pollard J, Mankoo PK, Henry C, Vincent L, Geslin C, Baltes N, Bergstrom DA. Differential antitumor activity of aflibercept and bevacizumab in patient-derived xenograft models of colorectal cancer. *Mol Cancer Ther* 2014; **13**: 1636-1644 [PMID: 24688047 DOI: 10.1158/1535-7163.MCT-13-0753]

42 **Goede V**, Coutelle O, Neuneier J, Reinacher-Schick A, Schnell R, Koslowsky TC, Weihrauch MR, Cremer B, Kashkar H, Odenthal M, Augustin HG, Schmiegel W, Hallek M, Hacker UT. Identification of serum angiopoietin-2 as a biomarker for clinical outcome of colorectal cancer patients treated with bevacizumab-containing therapy. *Br J Cancer* 2010; **103**: 1407-1414 [PMID: 20924372 DOI: 10.1038/sj.bjc.6605925]

43 **Scholz A**, Harter PN, Cremer S, Yalcin BH, Gurnik S, Yamaji M, Di Tacchio M, Sommer K, Baumgarten P, Bähr O, Steinbach JP, Trojan J, Glas M, Herrlinger U, Krex D, Meinhardt M, Weyerbrock A, Timmer M, Goldbrunner R, Deckert M, Braun C, Schittenhelm J, Frueh JT, Ullrich E, Mittelbronn M, Plate KH, Reiss Y. Endothelial cell-derived angiopoietin-2 is a therapeutic target in treatment-naive and bevacizumab-resistant glioblastoma. *EMBO Mol Med* 2016; **8**: 39-57 [PMID: 26666269 DOI: 10.15252/emmm.201505505]

44 **Rigamonti N**, Kadioglu E, Keklikoglou I, Wyser Rmili C, Leow CC, De Palma M. Role of angiopoietin-2 in adaptive tumor resistance to VEGF signaling blockade. *Cell Rep* 2014; **8**: 696-706 [PMID: 25088418 DOI: 10.1016/j.celrep.2014.06.059]

45 **Mitsuhashi A**, Goto H, Saijo A, Trung VT, Aono Y, Ogino H, Kuramoto T, Tabata S, Uehara H, Izumi K, Yoshida M, Kobayashi H, Takahashi H, Gotoh M, Kakiuchi S, Hanibuchi M, Yano S, Yokomise H, Sakiyama S, Nishioka Y. Fibrocyte-like cells mediate acquired resistance to anti-angiogenic therapy with bevacizumab. *Nat Commun* 2015; **6**: 8792 [PMID: 26635184 DOI: 10.1038/ncomms9792]

46 **Goto H**, Nishioka Y. Fibrocytes: A Novel Stromal Cells to Regulate Resistance to Anti-Angiogenic Therapy and Cancer Progression. *Int J Mol Sci* 2017; **19** [PMID: 29286323 DOI: 10.3390/ijms19010098]

47 **Jahangiri A**, De Lay M, Miller LM, Carbonell WS, Hu YL, Lu K, Tom MW, Paquette J, Tokuyasu TA, Tsao S, Marshall R, Perry A, Bjorgan KM, Chaumeil MM, Ronen SM, Bergers G, Aghi MK. Gene expression profile identifies tyrosine kinase c-Met as a targetable mediator of antiangiogenic therapy resistance. *Clin Cancer Res* 2013; **19**: 1773-1783 [PMID: 23307858 DOI: 10.1158/1078-0432.CCR-12-1281]

48 **Mutsaers AJ**, Francia G, Man S, Lee CR, Ebos JM, Wu Y, Witte L, Berry S, Moore M, Kerbel RS. Dose-dependent increases in circulating TGF-alpha and other EGFR ligands act as pharmacodynamic markers for optimal biological dosing of cetuximab and are tumor independent. *Clin Cancer Res* 2009; **15**: 2397-2405 [PMID: 19276250 DOI: 10.1158/1078-0432.CCR-08-1627]

49 **Loupakis F**, Cremolini C, Fioravanti A, Orlandi P, Salvatore L, Masi G, Schirripa M, Di Desidero T, Antoniotti C, Canu B, Faviana P, Sensi E, Lupi C, Fontanini G, Basolo F, Di Paolo A, Danesi R, Falcone A, Bocci G. EGFR ligands as pharmacodynamic biomarkers in metastatic colorectal cancer patients treated with cetuximab and irinotecan. *Target Oncol* 2014; **9**: 205-214 [PMID: 23821377 DOI: 10.1007/s11523-013-0284-7]

50 **Montagut C**, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, Salido M, Gallen M, Marsters S, Tsai SP, Minoche A, Seshagiri S, Serrano S, Himmelbauer H, Bellmunt J, Rovira A, Settleman J, Bosch F, Albanell J. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. *Nat Med* 2012; **18**: 221-223 [PMID: 22270724 DOI: 10.1038/nm.2609]

51 **Arena S**, Siravegna G, Mussolin B, Kearns JD, Wolf BB, Misale S, Lazzari L, Bertotti A, Trusolino L, Adjei AA, Montagut C, Di Nicolantonio F, Nering R, Bardelli A. MM-151 overcomes acquired resistance to cetuximab and panitumumab in colorectal cancers harboring EGFR extracellular domain mutations. *Sci Transl Med* 2016; **8**: 324ra14 [PMID: 26843189 DOI: 10.1126/scitranslmed.aad5640]

52 **Sánchez-Martín FJ**, Bellosillo B, Gelabert-Baldrich M, Dalmases A, Cañadas I, Vidal J, Martinez A, Argilés G, Siravegna G, Arena S, Koefoed K, Visa L, Arpí O, Horak ID, Iglesias M, Stroh C, Kragh M, Rovira A, Albanell J, Tabernero J, Bardelli A, Montagut C. The First-in-class Anti-EGFR Antibody Mixture Sym004 Overcomes Cetuximab Resistance Mediated by EGFR Extracellular Domain Mutations in Colorectal Cancer. *Clin Cancer Res* 2016; **22**: 3260-3267 [PMID: 26888827 DOI: 10.1158/1078-0432.CCR-15-2400]

53 **Dienstmann R**, Patnaik A, Garcia-Carbonero R, Cervantes A, Benavent M, Roselló S, Tops BB, van der Post RS, Argilés G, Skartved NJ, Hansen UH, Hald R, Pedersen MW, Kragh M, Horak ID, Braun S, Van Cutsem E, Tolcher AW, Tabernero J. Safety and Activity of the First-in-Class Sym004 Anti-EGFR Antibody Mixture in Patients with Refractory Colorectal Cancer. *Cancer Discov* 2015; **5**: 598-609 [PMID: 25962717 DOI: 10.1158/2159-8290.CD-14-1432]

54 **Montagut C**, Argilés G, Ciardiello F, Poulsen TT, Dienstmann R, Kragh M, Kopetz S, Lindsted T, Ding C, Vidal J, Clausell-Tormos J, Siravegna G, Sánchez-Martín FJ, Koefoed K, Pedersen MW, Grandal MM, Dvorkin M, Wyrwicz L, Rovira A, Cubillo A, Salazar R, Desseigne F, Nadal C, Albanell J, Zagonel V, Siena S, Fumi G, Rospo G, Nadler P, Horak ID, Bardelli A, Tabernero J. Efficacy of Sym004 in Patients With Metastatic Colorectal Cancer With Acquired Resistance to Anti-EGFR Therapy and Molecularly Selected by Circulating Tumor DNA Analyses: A Phase 2 Randomized Clinical Trial. *JAMA Oncol* 2018; **4**: e175245 [PMID: 29423521 DOI: 10.1001/jamaoncol.2017.5245]

55 **Bagchi A**, Haidar JN, Eastman SW, Vieth M, Topper M, Iacolina MD, Walker JM, Forest A, Shen Y, Novosiadly RD, Ferguson KM. Molecular Basis for Necitumumab Inhibition of EGFR Variants Associated with Acquired Cetuximab Resistance. *Mol Cancer Ther* 2018; **17**: 521-531 [PMID: 29158469 DOI: 10.1158/1535-7163.MCT-17-0575]

56 **Elez E**, Hendlisz A, Delaunoit T, Sastre J, Cervantes A, Varea R, Chao G, Wallin J, Tabernero J. Phase II study of necitumumab plus modified FOLFOX6 as first-line treatment in patients with locally advanced or metastatic colorectal cancer. *Br J Cancer* 2016; **114**: 372-380 [PMID: 26766738 DOI: 10.1038/bjc.2015.480]

57 **Van Cutsem E**, Köhne CH, Láng I, Folprecht G, Nowacki MP, Cascinu S, Shchepotin I, Maurel J, Cunningham D, Tejpar S, Schlichting M, Zubel A, Celik I, Rougier P, Ciardiello F. 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**: 2011-2019 [PMID: 21502544 DOI: 10.1200/JCO.2010.33.5091]

58 **Amado RG**, Wolf M, Peeters M, Van Cutsem E, Siena S, Freeman DJ, Juan T, Sikorski R, Suggs S, Radinsky R, Patterson SD, Chang DD. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol* 2008; **26**: 1626-1634 [PMID: 18316791 DOI: 10.1200/JCO.2007.14.7116]

59 **Misale S**, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov* 2014; **4**: 1269-1280 [PMID: 25293556 DOI: 10.1158/2159-8290.CD-14-0462]

60 **Van Cutsem E**, Lenz HJ, Köhne CH, Heinemann V, Tejpar S, Melezínek I, Beier F, Stroh C, Rougier P, van Krieken JH, Ciardiello F. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol* 2015; **33**: 692-700 [PMID: 25605843 DOI: 10.1200/JCO.2014.59.4812]

61 **De Roock W**, Jonker DJ, Di Nicolantonio F, Sartore-Bianchi A, Tu D, Siena S, Lamba S, Arena S, Frattini M, Piessevaux H, Van Cutsem E, O'Callaghan CJ, Khambata-Ford S, Zalcberg JR, Simes J, Karapetis CS, Bardelli A, Tejpar S. Association of KRAS p.G13D mutation with outcome in patients with chemotherapy-refractory metastatic colorectal cancer treated with cetuximab. *JAMA* 2010; **304**: 1812-1820 [PMID: 20978259 DOI: 10.1001/jama.2010.1535]

62 **Peeters M**, Douillard JY, Van Cutsem E, Siena S, Zhang K, Williams R, Wiezorek J. Mutant KRAS codon 12 and 13 alleles in patients with metastatic colorectal cancer: assessment as prognostic and predictive biomarkers of response to panitumumab. *J Clin Oncol* 2013; **31**: 759-765 [PMID: 23182985 DOI: 10.1200/JCO.2012.45.1492]

63 **Schirripa M**, Loupakis F, Lonardi S, Cremolini C, Bergamo F, Zagonel V, Falcone A. Phase II study of single-agent cetuximab in KRAS G13D mutant metastatic colorectal cancer. *Ann Oncol* 2015; **26**: 2503 [PMID: 26371285 DOI: 10.1093/annonc/mdv385]

64 **Ryan MB**, Corcoran RB. Therapeutic strategies to target RAS-mutant cancers. *Nat Rev Clin Oncol* 2018; **15**: 709-720 [PMID: 30275515 DOI: 10.1038/s41571-018-0105-0]

65 **Simanshu DK**, Nissley DV, McCormick F. RAS Proteins and Their Regulators in Human Disease. *Cell* 2017; **170**: 17-33 [PMID: 28666118 DOI: 10.1016/j.cell.2017.06.009]

66 **Spira AI**, Riely GJ, Gadgeel SM. KRYSTAL-1: Activity and safety of adagrasib (MRTX849) in patients with advanced/metastatic non-small cell lung cancer (NSCLC) harboring a KRASG12C mutation. *J of Clin Oncol* 2022; **40**: 9002 [DOI: 10.1200/jco.2022.40.16\_suppl.9002]

67 **Hong DS**, Fakih MG, Strickler JH, Desai J, Durm GA, Shapiro GI, Falchook GS, Price TJ, Sacher A, Denlinger CS, Bang YJ, Dy GK, Krauss JC, Kuboki Y, Kuo JC, Coveler AL, Park K, Kim TW, Barlesi F, Munster PN, Ramalingam SS, Burns TF, Meric-Bernstam F, Henary H, Ngang J, Ngarmchamnanrith G, Kim J, Houk BE, Canon J, Lipford JR, Friberg G, Lito P, Govindan R, Li BT. KRAS(G12C) Inhibition with Sotorasib in Advanced Solid Tumors. *N Engl J Med* 2020; **383**: 1207-1217 [PMID: 32955176 DOI: 10.1056/NEJMoa1917239]

68 **Visscher M**, Arkin MR, Dansen TB. Covalent targeting of acquired cysteines in cancer. *Curr Opin Chem Biol* 2016; **30**: 61-67 [PMID: 26629855 DOI: 10.1016/j.cbpa.2015.11.004]

69 **Hallin J**, Engstrom LD, Hargis L, Calinisan A, Aranda R, Briere DM, Sudhakar N, Bowcut V, Baer BR, Ballard JA, Burkard MR, Fell JB, Fischer JP, Vigers GP, Xue Y, Gatto S, Fernandez-Banet J, Pavlicek A, Velastagui K, Chao RC, Barton J, Pierobon M, Baldelli E, Patricoin EF 3rd, Cassidy DP, Marx MA, Rybkin II, Johnson ML, Ou SI, Lito P, Papadopoulos KP, Jänne PA, Olson P, Christensen JG. The KRAS(G12C) Inhibitor MRTX849 Provides Insight toward Therapeutic Susceptibility of KRAS-Mutant Cancers in Mouse Models and Patients. *Cancer Discov* 2020; **10**: 54-71 [PMID: 31658955 DOI: 10.1158/2159-8290.CD-19-1167]

70 **US** **National Library of Medicine**. Phase 3 Study of MRTX849 With Cetuximab vs Chemotherapy in Patients With Advanced Colorectal Cancer With KRAS G12C Mutation (KRYSTAL-10). Mar 11, 2021. Available from: https://clinicaltrials.gov/ct2/show/NCT04793958

71 **Dienstmann R**, Connor K, Byrne AT; COLOSSUS Consortium. Precision Therapy in RAS Mutant Colorectal Cancer. *Gastroenterology* 2020; **158**: 806-811 [PMID: 31972237 DOI: 10.1053/j.gastro.2019.12.051]

72 **Wang X**, Allen S, Blake JF, Bowcut V, Briere DM, Calinisan A, Dahlke JR, Fell JB, Fischer JP, Gunn RJ, Hallin J, Laguer J, Lawson JD, Medwid J, Newhouse B, Nguyen P, O'Leary JM, Olson P, Pajk S, Rahbaek L, Rodriguez M, Smith CR, Tang TP, Thomas NC, Vanderpool D, Vigers GP, Christensen JG, Marx MA. Identification of MRTX1133, a Noncovalent, Potent, and Selective KRAS(G12D) Inhibitor. *J Med Chem* 2022; **65**: 3123-3133 [PMID: 34889605 DOI: 10.1021/acs.jmedchem.1c01688]

73 **Knox JE**, Jiang J, Burnett GL, Liu Y, Weller CE, Wang ZC, McDowell L, Steele SL, Chin S, Chou KJ, Wang F, Zhong MQ, Koltun ES, Wildes D, Singh M, Gill AL, Smith JA. Abstract 3596: RM-036, a first-in-class, orally-bioavailable, Tri-Complex covalent KRASG12D(ON) inhibitor, drives profound anti-tumor activity in KRASG12D mutant tumor models, Cancer Research. 2022. https://aacrjournals.org/cancerres/article/82/12\_Supplement/3596/702321/Abstract-3596-RM-036-a-first-in-class-orally

74 **Hofmann MH**, Gerlach D, Misale S, Petronczki M, Kraut N. Expanding the Reach of Precision Oncology by Drugging All KRAS Mutants. *Cancer Discov* 2022; **12**: 924-937 [PMID: 35046095 DOI: 10.1158/2159-8290.CD-21-1331]

75 **Tolcher AW**, Peng W, Calvo E. Rational Approaches for Combination Therapy Strategies Targeting the MAP Kinase Pathway in Solid Tumors. *Mol Cancer Ther* 2018; **17**: 3-16 [PMID: 29295962 DOI: 10.1158/1535-7163.MCT-17-0349]

76 **Khokhlatchev AV**, Canagarajah B, Wilsbacher J, Robinson M, Atkinson M, Goldsmith E, Cobb MH. Phosphorylation of the MAP kinase ERK2 promotes its homodimerization and nuclear translocation. *Cell* 1998; **93**: 605-615 [PMID: 9604935 DOI: 10.1016/s0092-8674(00)81189-7]

77 **Infante JR**, Fecher LA, Falchook GS, Nallapareddy S, Gordon MS, Becerra C, DeMarini DJ, Cox DS, Xu Y, Morris SR, Peddareddigari VG, Le NT, Hart L, Bendell JC, Eckhardt G, Kurzrock R, Flaherty K, Burris HA 3rd, Messersmith WA. Safety, pharmacokinetic, pharmacodynamic, and efficacy data for the oral MEK inhibitor trametinib: a phase 1 dose-escalation trial. *Lancet Oncol* 2012; **13**: 773-781 [PMID: 22805291 DOI: 10.1016/S1470-2045(12)70270-X]

78 **Rosen LS**, LoRusso P, Ma WW, Goldman JW, Weise A, Colevas AD, Adjei A, Yazji S, Shen A, Johnston S, Hsieh HJ, Chan IT, Sikic BI. A first-in-human phase I study to evaluate the MEK1/2 inhibitor, cobimetinib, administered daily in patients with advanced solid tumors. *Invest New Drugs* 2016; **34**: 604-613 [PMID: 27424159 DOI: 10.1007/s10637-016-0374-3]

79 **Chenard-Poirier M**, Kaiser M, Boyd K. Results from the biomarker-driven basket trial of RO5126766 (CH5127566), a potent RAF/MEK inhibitor, in RAS- or RAF-mutated malignancies including multiple myeloma. *J of Clin Onco* 2017; **35**: 2506-2506 [DOI: 10.1200/jco.2017.35.15\_suppl.2506]

80 **Bardelli A**, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. *J Clin Oncol* 2010; **28**: 1254-1261 [PMID: 20100961 DOI: 10.1200/JCO.2009.24.6116]

81 **Misale S**, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, Bencardino K, Cercek A, Chen CT, Veronese S, Zanon C, Sartore-Bianchi A, Gambacorta M, Gallicchio M, Vakiani E, Boscaro V, Medico E, Weiser M, Siena S, Di Nicolantonio F, Solit D, Bardelli A. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. *Nature* 2012; **486**: 532-536 [PMID: 22722830 DOI: 10.1038/nature11156]

82 **Deming DA**, Cavalcante LL, Lubner SJ, Mulkerin DL, LoConte NK, Eickhoff JC, Kolesar JM, Fioravanti S, Greten TF, Compton K, Doyle AG, Wilding G, Duffy A, Liu G. A phase I study of selumetinib (AZD6244/ARRY-142866), a MEK1/2 inhibitor, in combination with cetuximab in refractory solid tumors and KRAS mutant colorectal cancer. *Invest New Drugs* 2016; **34**: 168-175 [PMID: 26666244 DOI: 10.1007/s10637-015-0314-7]

83 **Ledys F**, Derangère V, Réda M, Guion JF, Milliex R, Roux V, Limagne E, Arnould L, Bengrine L, Ghiringhelli F, Rébé C. Anti-MEK and Anti-EGFR mAbs in RAS-Mutant Metastatic Colorectal Cancer: Case Series and Rationale. *Adv Ther* 2019; **36**: 1480-1484 [PMID: 30980281 DOI: 10.1007/s12325-019-00949-y]

84 **Lieu CH**, Hidalgo M, Berlin JD, Ko AH, Cervantes A, LoRusso P, Gerber DE, Eder JP, Eckhardt SG, Kapp AV, Tsuhako A, McCall B, Pirzkall A, Uyei A, Tabernero J. 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**: 1024-1e89 [PMID: 28592615 DOI: 10.1634/theoncologist.2017-0175]

85 **Huijberts SCFA**, van Geel RMJM, van Brummelen EMJ, Opdam FL, Marchetti S, Steeghs N, Pulleman S, Thijssen B, Rosing H, Monkhorst K, Huitema ADR, Beijnen JH, Bernards R, Schellens JHM. Phase I study of lapatinib plus trametinib in patients with KRAS-mutant colorectal, non-small cell lung, and pancreatic cancer. *Cancer Chemother Pharmacol* 2020; **85**: 917-930 [PMID: 32274564 DOI: 10.1007/s00280-020-04066-4]

86 **Lee MS**, Helms TL, Feng N, Gay J, Chang QE, Tian F, Wu JY, Toniatti C, Heffernan TP, Powis G, Kwong LN, Kopetz S. Efficacy of the combination of MEK and CDK4/6 inhibitors in vitro and in vivo in KRAS mutant colorectal cancer models. *Oncotarget* 2016; **7**: 39595-39608 [PMID: 27167191 DOI: 10.18632/oncotarget.9153]

87 **Ziemke EK**, Dosch JS, Maust JD, Shettigar A, Sen A, Welling TH, Hardiman KM, Sebolt-Leopold JS. Sensitivity of KRAS-Mutant Colorectal Cancers to Combination Therapy That Cotargets MEK and CDK4/6. *Clin Cancer Res* 2016; **22**: 405-414 [PMID: 26369631 DOI: 10.1158/1078-0432.CCR-15-0829]

88 **Bailly C**, Thuru X, Quesnel B. Combined cytotoxic chemotherapy and immunotherapy of cancer: modern times. *NAR Cancer* 2020; **2**: zcaa002 [PMID: 34316682 DOI: 10.1093/narcan/zcaa002]

89 **Canon J**, Rex K, Saiki AY, Mohr C, Cooke K, Bagal D, Gaida K, Holt T, Knutson CG, Koppada N, Lanman BA, Werner J, Rapaport AS, San Miguel T, Ortiz R, Osgood T, Sun JR, Zhu X, McCarter JD, Volak LP, Houk BE, Fakih MG, O'Neil BH, Price TJ, Falchook GS, Desai J, Kuo J, Govindan R, Hong DS, Ouyang W, Henary H, Arvedson T, Cee VJ, Lipford JR. The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 2019; **575**: 217-223 [PMID: 31666701 DOI: 10.1038/s41586-019-1694-1]

90 **Briere DM**, Li S, Calinisan A, Sudhakar N, Aranda R, Hargis L, Peng DH, Deng J, Engstrom LD, Hallin J, Gatto S, Fernandez-Banet J, Pavlicek A, Wong KK, Christensen JG, Olson P. The KRAS(G12C) Inhibitor MRTX849 Reconditions the Tumor Immune Microenvironment and Sensitizes Tumors to Checkpoint Inhibitor Therapy. *Mol Cancer Ther* 2021; **20**: 975-985 [PMID: 33722854 DOI: 10.1158/1535-7163.MCT-20-0462]

91 **Hutton JE**, Wang X, Zimmerman LJ, Slebos RJ, Trenary IA, Young JD, Li M, Liebler DC. Oncogenic KRAS and BRAF Drive Metabolic Reprogramming in Colorectal Cancer. *Mol Cell Proteomics* 2016; **15**: 2924-2938 [PMID: 27340238 DOI: 10.1074/mcp.M116.058925]

92 **Gouw AM**, Eberlin LS, Margulis K, Sullivan DK, Toal GG, Tong L, Zare RN, Felsher DW. Oncogene KRAS activates fatty acid synthase, resulting in specific ERK and lipid signatures associated with lung adenocarcinoma. *Proc Natl Acad Sci U S A* 2017; **114**: 4300-4305 [PMID: 28400509 DOI: 10.1073/pnas.1617709114]

93 **Berkovic MC**, Mikulic D, Bilic-Curcic I, Mrzljak A. How far along are we in revealing the connection between metformin and colorectal cancer? *World J Gastroenterol* 2021; **27**: 1362-1368 [PMID: 33911461 DOI: 10.3748/wjg.v27.i14.1362]

94 **Richard SM**, Martinez Marignac VL. Sensitization to oxaliplatin in HCT116 and HT29 cell lines by metformin and ribavirin and differences in response to mitochondrial glutaminase inhibition. *J Cancer Res Ther* 2015; **11**: 336-340 [PMID: 26148596 DOI: 10.4103/0973-1482.157317]

95 **Vernieri C**, Galli F, Ferrari L, Marchetti P, Lonardi S, Maiello E, Iaffaioli RV, Zampino MG, Zaniboni A, De Placido S, Banzi M, Damiani A, Ferrari D, Rosati G, Labianca RF, Bidoli P, Frassineti GL, Nicolini M, Pavesi L, Tronconi MC, Buonadonna A, Ferrario S, Re GL, Adamo V, Tamburini E, Clerico M, Giordani P, Leonardi F, Barni S, Ciarlo A, Cavanna L, Gori S, Cinieri S, Faedi M, Aglietta M, Antista M, Dotti KF, Galli F, Di Bartolomeo M; TOSCA (Three or Six Colon Adjuvant) Investigators. Impact of Metformin Use and Diabetic Status During Adjuvant Fluoropyrimidine-Oxaliplatin Chemotherapy on the Outcome of Patients with Resected Colon Cancer: A TOSCA Study Subanalysis. *Oncologist* 2019; **24**: 385-393 [PMID: 30606884 DOI: 10.1634/theoncologist.2018-0442]

96 **Singh PP**, Shi Q, Foster NR, Grothey A, Nair SG, Chan E, Shields AF, Goldberg RM, Gill S, Kahlenberg MS, Sinicrope FA, Sargent DJ, Alberts SR. Relationship Between Metformin Use and Recurrence and Survival in Patients With Resected Stage III Colon Cancer Receiving Adjuvant Chemotherapy: Results From North Central Cancer Treatment Group N0147 (Alliance). *Oncologist* 2016; **21**: 1509-1521 [PMID: 27881709 DOI: 10.1634/theoncologist.2016-0153]

97 **Spillane S**, Bennett K, Sharp L, Barron TI. A cohort study of metformin exposure and survival in patients with stage I-III colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2013; **22**: 1364-1373 [PMID: 23753040 DOI: 10.1158/1055-9965.EPI-13-0347]

98 **Lee GE**, Aung T, Lim KH. Examining the effects of metformin on survival outcome in stage II/III colorectal cancer patients with diabetes mellitus. *J of Clin Oncol* 2012; **30**: 3589-3589 [DOI: 10.1200/jco.2012.30.15\_suppl.3589]

99 **Yun J**, Mullarky E, Lu C, Bosch KN, Kavalier A, Rivera K, Roper J, Chio II, Giannopoulou EG, Rago C, Muley A, Asara JM, Paik J, Elemento O, Chen Z, Pappin DJ, Dow LE, Papadopoulos N, Gross SS, Cantley LC. Vitamin C selectively kills KRAS and BRAF mutant colorectal cancer cells by targeting GAPDH. *Science* 2015; **350**: 1391-1396 [PMID: 26541605 DOI: 10.1126/science.aaa5004]

100 **Wang F**, He MM, Wang ZX, Li S, Jin Y, Ren C, Shi SM, Bi BT, Chen SZ, Lv ZD, Hu JJ, Wang ZQ, Wang FH, Wang DS, Li YH, Xu RH. Phase I study of high-dose ascorbic acid with mFOLFOX6 or FOLFIRI in patients with metastatic colorectal cancer or gastric cancer. *BMC Cancer* 2019; **19**: 460 [PMID: 31096937 DOI: 10.1186/s12885-019-5696-z]

101 **Davies H**, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, Davis N, Dicks E, Ewing R, Floyd Y, Gray K, Hall S, Hawes R, Hughes J, Kosmidou V, Menzies A, Mould C, Parker A, Stevens C, Watt S, Hooper S, Wilson R, Jayatilake H, Gusterson BA, Cooper C, Shipley J, Hargrave D, Pritchard-Jones K, Maitland N, Chenevix-Trench G, Riggins GJ, Bigner DD, Palmieri G, Cossu A, Flanagan A, Nicholson A, Ho JW, Leung SY, Yuen ST, Weber BL, Seigler HF, Darrow TL, Paterson H, Marais R, Marshall CJ, Wooster R, Stratton MR, Futreal PA. Mutations of the BRAF gene in human cancer. *Nature* 2002; **417**: 949-954 [PMID: 12068308 DOI: 10.1038/nature00766]

102 **Yaeger R**, Chatila WK, Lipsyc MD. Clinical Sequencing Defines the Genomic Landscape of Metastatic Colorectal Cancer. *Cancer Cell* 2018; **33**: 125-136 [DOI: 10.1016/j.ccell.2017.12.004]

103 **Barras D**, Missiaglia E, Wirapati P, Sieber OM, Jorissen RN, Love C, Molloy PL, Jones IT, McLaughlin S, Gibbs P, Guinney J, Simon IM, Roth AD, Bosman FT, Tejpar S, Delorenzi M. BRAF V600E Mutant Colorectal Cancer Subtypes Based on Gene Expression. *Clin Cancer Res* 2017; **23**: 104-115 [PMID: 27354468 DOI: 10.1158/1078-0432.CCR-16-0140]

104 **Wan PT**, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, Jones CM, Marshall CJ, Springer CJ, Barford D, Marais R; Cancer Genome Project. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004; **116**: 855-867 [PMID: 15035987 DOI: 10.1016/s0092-8674(04)00215-6]

105 **Clarke CN**, Kopetz ES. BRAF mutant colorectal cancer as a distinct subset of colorectal cancer: clinical characteristics, clinical behavior, and response to targeted therapies. *J Gastrointest Oncol* 2015; **6**: 660-667 [PMID: 26697199 DOI: 10.3978/j.issn.2078-6891.2015.077]

106 **Sinicrope FA**, Shi Q, Smyrk TC, Thibodeau SN, Dienstmann R, Guinney J, Bot BM, Tejpar S, Delorenzi M, Goldberg RM, Mahoney M, Sargent DJ, Alberts SR. Molecular markers identify subtypes of stage III colon cancer associated with patient outcomes. *Gastroenterology* 2015; **148**: 88-99 [PMID: 25305506 DOI: 10.1053/j.gastro.2014.09.041]

107 **Jones JC**, Renfro LA, Al-Shamsi HO, Schrock AB, Rankin A, Zhang BY, Kasi PM, Voss JS, Leal AD, Sun J, Ross J, Ali SM, Hubbard JM, Kipp BR, McWilliams RR, Kopetz S, Wolff RA, Grothey A. (Non-V600) BRAF Mutations Define a Clinically Distinct Molecular Subtype of Metastatic Colorectal Cancer. *J Clin Oncol* 2017; **35**: 2624-2630 [PMID: 28486044 DOI: 10.1200/JCO.2016.71.4394]

108 **Sinicrope FA**, Mahoney MR, Smyrk TC, Thibodeau SN, Warren RS, Bertagnolli MM, Nelson GD, Goldberg RM, Sargent DJ, Alberts SR. Prognostic impact of deficient DNA mismatch repair in patients with stage III colon cancer from a randomized trial of FOLFOX-based adjuvant chemotherapy. *J Clin Oncol* 2013; **31**: 3664-3672 [PMID: 24019539 DOI: 10.1200/JCO.2013.48.9591]

109 **Tran B**, Kopetz S, Tie J, Gibbs P, Jiang ZQ, Lieu CH, Agarwal A, Maru DM, Sieber O, Desai J. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer* 2011; **117**: 4623-4632 [PMID: 21456008 DOI: 10.1002/cncr.26086]

110 **Carethers JM**, Jung BH. Genetics and Genetic Biomarkers in Sporadic Colorectal Cancer. *Gastroenterology* 2015; **149**: 1177-1190.e3 [PMID: 26216840 DOI: 10.1053/j.gastro.2015.06.047]

111 **Tie J**, Gibbs P, Lipton L, Christie M, Jorissen RN, Burgess AW, Croxford M, Jones I, Langland R, Kosmider S, McKay D, Bollag G, Nolop K, Sieber OM, Desai J. Optimizing targeted therapeutic development: analysis of a colorectal cancer patient population with the BRAF(V600E) mutation. *Int J Cancer* 2011; **128**: 2075-2084 [PMID: 20635392 DOI: 10.1002/ijc.25555]

112 **Deng G**, Bell I, Crawley S. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. *Clin Cancer Res* 2004; **10**: 191-195 [DOI: 10.1158/1078-0432.ccr-1118-3]

113 **Wang Y**, Jones JC, Kipp BR, Grothey A. Activity of EGFR antibody in non-V600 BRAF mutant metastatic colorectal cancer. *Ann Oncol* 2019; **30**: 147-149 [PMID: 30364934 DOI: 10.1093/annonc/mdy477]

114 **Yaeger R**, Kotani D, Mondaca S, Parikh AR, Bando H, Van Seventer EE, Taniguchi H, Zhao H, Thant CN, de Stanchina E, Rosen N, Corcoran RB, Yoshino T, Yao Z, Ebi H. Response to Anti-EGFR Therapy in Patients with BRAF non-V600-Mutant Metastatic Colorectal Cancer. *Clin Cancer Res* 2019; **25**: 7089-7097 [PMID: 31515458 DOI: 10.1158/1078-0432.CCR-19-2004]

115 **Loupakis F**, Cremolini C, Masi G, Lonardi S, Zagonel V, Salvatore L, Cortesi E, Tomasello G, Ronzoni M, Spadi R, Zaniboni A, Tonini G, Buonadonna A, Amoroso D, Chiara S, Carlomagno C, Boni C, Allegrini G, Boni L, Falcone A. Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med* 2014; **371**: 1609-1618 [PMID: 25337750 DOI: 10.1056/NEJMoa1403108]

116 **Cremolini C**, Antoniotti C, Rossini D, Lonardi S, Loupakis F, Pietrantonio F, Bordonaro R, Latiano TP, Tamburini E, Santini D, Passardi A, Marmorino F, Grande R, Aprile G, Zaniboni A, Murgioni S, Granetto C, Buonadonna A, Moretto R, Corallo S, Cordio S, Antonuzzo L, Tomasello G, Masi G, Ronzoni M, Di Donato S, Carlomagno C, Clavarezza M, Ritorto G, Mambrini A, Roselli M, Cupini S, Mammoliti S, Fenocchio E, Corgna E, Zagonel V, Fontanini G, Ugolini C, Boni L, Falcone A; GONO Foundation Investigators. Upfront FOLFOXIRI plus bevacizumab and reintroduction after progression versus mFOLFOX6 plus bevacizumab followed by FOLFIRI plus bevacizumab in the treatment of patients with metastatic colorectal cancer (TRIBE2): a multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol* 2020; **21**: 497-507 [PMID: 32164906 DOI: 10.1016/S1470-2045(19)30862-9]

117 **Cremolini C**, Antoniotti C, Stein A, Bendell J, Gruenberger T, Rossini D, Masi G, Ongaro E, Hurwitz H, Falcone A, Schmoll HJ, Di Maio M. Individual Patient Data Meta-Analysis of FOLFOXIRI Plus Bevacizumab Versus Doublets Plus Bevacizumab as Initial Therapy of Unresectable Metastatic Colorectal Cancer. *J Clin Oncol* 2020: JCO2001225 [PMID: 32816630 DOI: 10.1200/JCO.20.01225]

118 **Chapman PB**, Hauschild A, Robert C, Haanen JB, Ascierto P, Larkin J, Dummer R, Garbe C, Testori A, Maio M, Hogg D, Lorigan P, Lebbe C, Jouary T, Schadendorf D, Ribas A, O'Day SJ, Sosman JA, Kirkwood JM, Eggermont AM, Dreno B, Nolop K, Li J, Nelson B, Hou J, Lee RJ, Flaherty KT, McArthur GA; BRIM-3 Study Group. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; **364**: 2507-2516 [PMID: 21639808 DOI: 10.1056/NEJMoa1103782]

119 **Sosman JA**, Kim KB, Schuchter L, Gonzalez R, Pavlick AC, Weber JS, McArthur GA, Hutson TE, Moschos SJ, Flaherty KT, Hersey P, Kefford R, Lawrence D, Puzanov I, Lewis KD, Amaravadi RK, Chmielowski B, Lawrence HJ, Shyr Y, Ye F, Li J, Nolop KB, Lee RJ, Joe AK, Ribas A. Survival in BRAF V600-mutant advanced melanoma treated with vemurafenib. *N Engl J Med* 2012; **366**: 707-714 [PMID: 22356324 DOI: 10.1056/NEJMoa1112302]

120 **Kopetz S**, Desai J, Chan E. PLX4032 in metastatic colorectal cancer patients with mutant BRAF tumors. *J of Clin Oncol* 2010; **28**: 3534-3534 [DOI: 10.1200/jco.2010.28.15\_suppl.3534]

121 **Mao M**, Tian F, Mariadason JM, Tsao CC, Lemos R Jr, Dayyani F, Gopal YN, Jiang ZQ, Wistuba II, Tang XM, Bornman WG, Bollag G, Mills GB, Powis G, Desai J, Gallick GE, Davies MA, Kopetz S. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. *Clin Cancer Res* 2013; **19**: 657-667 [PMID: 23251002 DOI: 10.1158/1078-0432.CCR-11-1446]

122 **Prahallad A**, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R. Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; **483**: 100-103 [PMID: 22281684 DOI: 10.1038/nature10868]

123 **Corcoran RB**, Ebi H, Turke AB, Coffee EM, Nishino M, Cogdill AP, Brown RD, Della Pelle P, Dias-Santagata D, Hung KE, Flaherty KT, Piris A, Wargo JA, Settleman J, Mino-Kenudson M, Engelman JA. EGFR-mediated re-activation of MAPK signaling contributes to insensitivity of BRAF mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2012; **2**: 227-235 [PMID: 22448344 DOI: 10.1158/2159-8290.CD-11-0341]

124 **Yaeger R**, Cercek A, O'Reilly EM. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res* 2015; **21**: 1313-1320 [DOI: 10.1158/1078-0432.CCR-14-2779]

125 **Hong DS**, Morris VK, El Osta B, Sorokin AV, Janku F, Fu S, Overman MJ, Piha-Paul S, Subbiah V, Kee B, Tsimberidou AM, Fogelman D, Bellido J, Shureiqi I, Huang H, Atkins J, Tarcic G, Sommer N, Lanman R, Meric-Bernstam F, Kopetz S. Phase IB Study of Vemurafenib in Combination with Irinotecan and Cetuximab in Patients with Metastatic Colorectal Cancer with BRAFV600E Mutation. *Cancer Discov* 2016; **6**: 1352-1365 [PMID: 27729313 DOI: 10.1158/2159-8290.CD-16-0050]

126 **Kopetz S**, Guthrie KA, Morris VK, Lenz HJ, Magliocco AM, Maru D, Yan Y, Lanman R, Manyam G, Hong DS, Sorokin A, Atreya CE, Diaz LA, Allegra C, Raghav KP, Wang SE, Lieu CH, McDonough SL, Philip PA, Hochster HS. Randomized Trial of Irinotecan and Cetuximab With or Without Vemurafenib in BRAF-Mutant Metastatic Colorectal Cancer (SWOG S1406). *J Clin Oncol* 2021; **39**: 285-294 [PMID: 33356422 DOI: 10.1200/JCO.20.01994]

127 **Benson AB**, Venook AP, Al-Hawary MM, Cederquist L, Chen YJ, Ciombor KK, Cohen S, Cooper HS, Deming D, Engstrom PF, Garrido-Laguna I, Grem JL, Grothey A, Hochster HS, Hoffe S, Hunt S, Kamel A, Kirilcuk N, Krishnamurthi S, Messersmith WA, Meyerhardt J, Miller ED, Mulcahy MF, Murphy JD, Nurkin S, Saltz L, Sharma S, Shibata D, Skibber JM, Sofocleous CT, Stoffel EM, Stotsky-Himelfarb E, Willett CG, Wuthrick E, Gregory KM, Freedman-Cass DA. NCCN Guidelines Insights: Colon Cancer, Version 2.2018. *J Natl Compr Canc Netw* 2018; **16**: 359-369 [PMID: 29632055 DOI: 10.6004/jnccn.2018.0021]

128 **Elez E**, Ros J, Fernández J, Villacampa G, Moreno-Cárdenas AB, Arenillas C, Bernatowicz K, Comas R, Li S, Kodack DP, Fasani R, Garcia A, Gonzalo-Ruiz J, Piris-Gimenez A, Nuciforo P, Kerr G, Intini R, Montagna A, Germani MM, Randon G, Vivancos A, Smits R, Graus D, Perez-Lopez R, Cremolini C, Lonardi S, Pietrantonio F, Dienstmann R, Tabernero J, Toledo RA. RNF43 mutations predict response to anti-BRAF/EGFR combinatory therapies in BRAF(V600E) metastatic colorectal cancer. *Nat Med* 2022; **28**: 2162-2170 [PMID: 36097219 DOI: 10.1038/s41591-022-01976-z]

129 **Van Cutsem E**, Huijberts S, Grothey A, Yaeger R, Cuyle PJ, Elez E, Fakih M, Montagut C, Peeters M, Yoshino T, Wasan H, Desai J, Ciardiello F, Gollerkeri A, Christy-Bittel J, Maharry K, Sandor V, Schellens JHM, Kopetz S, Tabernero J. 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 Cancer Study. *J Clin Oncol* 2019; **37**: 1460-1469 [PMID: 30892987 DOI: 10.1200/JCO.18.02459]

130 **Corcoran RB**, André T, Atreya CE, Schellens JHM, Yoshino T, Bendell JC, Hollebecque A, McRee AJ, Siena S, Middleton G, Muro K, Gordon MS, Tabernero J, Yaeger R, O'Dwyer PJ, Humblet Y, De Vos F, Jung AS, Brase JC, Jaeger S, Bettinger S, Mookerjee B, Rangwala F, Van Cutsem E. Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAF(V600E)-Mutant Colorectal Cancer. *Cancer Discov* 2018; **8**: 428-443 [PMID: 29431699 DOI: 10.1158/2159-8290.CD-17-1226]

131 **Ros J**, Saoudi N, Baraibar I, Salva F, Tabernero J, Elez E. Encorafenib plus cetuximab for the treatment of BRAF-V600E-mutated metastatic colorectal cancer. *Therap Adv Gastroenterol* 2022; **15**: 17562848221110644 [PMID: 35812780 DOI: 10.1177/17562848221110644]

132 **Kopetz S**, Grothey A, Cutsem EV. Encorafenib plus cetuximab with or without binimetinib for BRAF V600E-mutant metastatic colorectal cancer: Quality-of-life results from a randomized, three-arm, phase III study vs the choice of either irinotecan or FOLFIRI plus cetuximab (BEACON CRC). *J of Clin Oncol* 2020; **38**: 8-8 [DOI: 10.1200/JCO.2020.38.4\_suppl.8]

133 **Grothey A**, Fakih M, Tabernero J. Management of BRAF-mutant metastatic colorectal cancer: a review of treatment options and evidence-based guidelines. *Ann Oncol* 2021; **32**: 959-967 [PMID: 33836264 DOI: 10.1016/j.annonc.2021.03.206]

134 **Grothey A**, Tabernero J, Taieb J. LBA-5 ANCHOR CRC: a single-arm, phase 2 study of encorafenib, binimetinib plus cetuximab in previously untreated BRAF V600E-mutant metastatic colorectal cancer. *Ann Oncol* 2020; **31**

135 **Corcoran RB**, André T, Yoshino T. Efficacy and circulating tumor DNA (ctDNA) analysis of the BRAF inhibitor dabrafenib (D), MEK inhibitor trametinib (T), and anti-EGFR antibody panitumumab (P) in patients (pts) with BRAF V600E-mutated (BRAFm) metastatic colorectal cancer (mCRC). *Ann Oncol* 2016: **27**

136 **Corcoran RB**, Atreya CE, Falchook GS, Kwak EL, Ryan DP, Bendell JC, Hamid O, Messersmith WA, Daud A, Kurzrock R, Pierobon M, Sun P, Cunningham E, Little S, Orford K, Motwani M, Bai Y, Patel K, Venook AP, Kopetz S. Combined BRAF and MEK Inhibition With Dabrafenib and Trametinib in BRAF V600-Mutant Colorectal Cancer. *J Clin Oncol* 2015; **33**: 4023-4031 [PMID: 26392102 DOI: 10.1200/JCO.2015.63.2471]

137 **Mao C**, Yang ZY, Hu XF, Chen Q, Tang JL. PIK3CA exon 20 mutations as a potential biomarker for resistance to anti-EGFR monoclonal antibodies in KRAS wild-type metastatic colorectal cancer: a systematic review and meta-analysis. *Ann Oncol* 2012; **23**: 1518-1525 [PMID: 22039088 DOI: 10.1093/annonc/mdr464]

138 **Chan AT**, Ogino S, Fuchs CS. Aspirin use and survival after diagnosis of colorectal cancer. *JAMA* 2009; **302**: 649-658 [PMID: 19671906 DOI: 10.1001/jama.2009.1112]

139 **Liao X**, Lochhead P, Nishihara R, Morikawa T, Kuchiba A, Yamauchi M, Imamura Y, Qian ZR, Baba Y, Shima K, Sun R, Nosho K, Meyerhardt JA, Giovannucci E, Fuchs CS, Chan AT, Ogino S. Aspirin use, tumor PIK3CA mutation, and colorectal-cancer survival. *N Engl J Med* 2012; **367**: 1596-1606 [PMID: 23094721 DOI: 10.1056/NEJMoa1207756]

140 **Siena S**, Sartore-Bianchi A, Di Nicolantonio F, Balfour J, Bardelli A. Biomarkers predicting clinical outcome of epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer. *J Natl Cancer Inst* 2009; **101**: 1308-1324 [PMID: 19738166 DOI: 10.1093/jnci/djp280]

141 **Bertotti A**, Migliardi G, Galimi F, Sassi F, Torti D, Isella C, Corà D, Di Nicolantonio F, Buscarino M, Petti C, Ribero D, Russolillo N, Muratore A, Massucco P, Pisacane A, Molinaro L, Valtorta E, Sartore-Bianchi A, Risio M, Capussotti L, Gambacorta M, Siena S, Medico E, Sapino A, Marsoni S, Comoglio PM, Bardelli A, Trusolino L. 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**: 508-523 [PMID: 22586653 DOI: 10.1158/2159-8290.CD-11-0109]

142 **Sclafani F**, Roy A, Cunningham D, Wotherspoon A, Peckitt C, Gonzalez de Castro D, Tabernero J, Glimelius B, Cervantes A, Eltahir Z, Oates J, Chau I. HER2 in high-risk rectal cancer patients treated in EXPERT-C, a randomized phase II trial of neoadjuvant capecitabine and oxaliplatin (CAPOX) and chemoradiotherapy (CRT) with or without cetuximab. *Ann Oncol* 2013; **24**: 3123-3128 [PMID: 24146218 DOI: 10.1093/annonc/mdt408]

143 **Seo AN**, Kwak Y, Kim DW, Kang SB, Choe G, Kim WH, Lee HS. HER2 status in colorectal cancer: its clinical significance and the relationship between HER2 gene amplification and expression. *PLoS One* 2014; **9**: e98528 [PMID: 24879338]

144 **Loree JM**, Bailey AM, Johnson AM, Yu Y, Wu W, Bristow CA, Davis JS, Shaw KR, Broaddus R, Banks KC, Lanman RB, Meric-Bernstam F, Overman MJ, Kopetz S, Raghav K. Molecular Landscape of ERBB2/ERBB3 Mutated Colorectal Cancer. *J Natl Cancer Inst* 2018; **110**: 1409-1417 [PMID: 29718453 DOI: 10.1093/jnci/djy067]

145 **Kloth M**, Ruesseler V, Engel C, Koenig K, Peifer M, Mariotti E, Kuenstlinger H, Florin A, Rommerscheidt-Fuss U, Koitzsch U, Wodtke C, Ueckeroth F, Holzapfel S, Aretz S, Propping P, Loeffler M, Merkelbach-Bruse S, Odenthal M, Friedrichs N, Heukamp LC, Zander T, Buettner R. Activating ERBB2/HER2 mutations indicate susceptibility to pan-HER inhibitors in Lynch and Lynch-like colorectal cancer. *Gut* 2016; **65**: 1296-1305 [PMID: 26001389 DOI: 10.1136/gutjnl-2014-309026]

146 **Ramanathan RK**, Hwang JJ, Zamboni WC, Sinicrope FA, Safran H, Wong MK, Earle M, Brufsky A, Evans T, Troetschel M, Walko C, Day R, Chen HX, Finkelstein S. Low overexpression of HER-2/neu in advanced colorectal cancer limits the usefulness of trastuzumab (Herceptin) and irinotecan as therapy. A phase II trial. *Cancer Invest* 2004; **22**: 858-865 [PMID: 15641483 DOI: 10.1081/cnv-200039645]

147 **Greally M**, Kelly CM, Cercek A. HER2: An emerging target in colorectal cancer. *Curr Probl Cancer* 2018; **42**: 560-571 [PMID: 30100092 DOI: 10.1016/j.currproblcancer.2018.07.001]

148 **Sartore-Bianchi A**, Trusolino L, Martino C, Bencardino K, Lonardi S, Bergamo F, Zagonel V, Leone F, Depetris I, Martinelli E, Troiani T, Ciardiello F, Racca P, Bertotti A, Siravegna G, Torri V, Amatu A, Ghezzi S, Marrapese G, Palmeri L, Valtorta E, Cassingena A, Lauricella C, Vanzulli A, Regge D, Veronese S, Comoglio PM, Bardelli A, Marsoni S, Siena S. 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**: 738-746 [PMID: 27108243 DOI: 10.1016/S1470-2045(16)00150-9]

149 **Valtorta E**, Martino C, Sartore-Bianchi A, Penaullt-Llorca F, Viale G, Risio M, Rugge M, Grigioni W, Bencardino K, Lonardi S, Zagonel V, Leone F, Noe J, Ciardiello F, Pinto C, Labianca R, Mosconi S, Graiff C, Aprile G, Frau B, Garufi C, Loupakis F, Racca P, Tonini G, Lauricella C, Veronese S, Truini M, Siena S, Marsoni S, Gambacorta M. Assessment of a HER2 scoring system for colorectal cancer: results from a validation study. *Mod Pathol* 2015; **28**: 1481-1491 [PMID: 26449765 DOI: 10.1038/modpathol.2015.98]

150 **Sartore-Bianchi A**, Lonardi S, Martino C, Fenocchio E, Tosi F, Ghezzi S, Leone F, Bergamo F, Zagonel V, Ciardiello F, Ardizzoni A, Amatu A, Bencardino K, Valtorta E, Grassi E, Torri V, Bonoldi E, Sapino A, Vanzulli A, Regge D, Cappello G, Bardelli A, Trusolino L, Marsoni S, Siena S. Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. *ESMO Open* 2020; **5**: e000911 [PMID: 32988996 DOI: 10.1136/esmoopen-2020-000911]

151 **Meric-Bernstam F**, Hurwitz H, Raghav KPS, McWilliams RR, Fakih M, VanderWalde A, Swanton C, Kurzrock R, Burris H, Sweeney C, Bose R, Spigel DR, Beattie MS, Blotner S, Stone A, Schulze K, Cuchelkar V, Hainsworth J. 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**: 518-530 [PMID: 30857956 DOI: 10.1016/S1470-2045(18)30904-5]

152 **Narita Y**, Yoshimoto T, Namai T, Asakawa T, Kawakami S, Gower-Page C, Reyes-Rivera I, Patel A, Nakamura Y. Pertuzumab Plus Trastuzumab for Treatment-Refractory HER2-Amplified Metastatic Colorectal Cancer: Comparison of the MyPathway Trial With a Real-World External Control Arm. *JCO Clin Cancer Inform* 2022; **6**: e2200022 [PMID: 35649212 DOI: 10.1200/CCI.22.00022]

153 **Nakamura Y**, Okamoto W, Kato T, Esaki T, Kato K, Komatsu Y, Yuki S, Masuishi T, Nishina T, Ebi H, Sawada K, Taniguchi H, Fuse N, Nomura S, Fukui M, Matsuda S, Sakamoto Y, Uchigata H, Kitajima K, Kuramoto N, Asakawa T, Olsen S, Odegaard JI, Sato A, Fujii S, Ohtsu A, Yoshino T. Circulating tumor DNA-guided treatment with pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer: a phase 2 trial. *Nat Med* 2021; **27**: 1899-1903 [PMID: 34764486 DOI: 10.1038/s41591-021-01553-w]

154 **Siena S**, Di Bartolomeo M, Raghav K, Masuishi T, Loupakis F, Kawakami H, Yamaguchi K, Nishina T, Fakih M, Elez E, Rodriguez J, Ciardiello F, Komatsu Y, Esaki T, Chung K, Wainberg Z, Sartore-Bianchi A, Saxena K, Yamamoto E, Bako E, Okuda Y, Shahidi J, Grothey A, Yoshino T; DESTINY-CRC01 investigators. Trastuzumab deruxtecan (DS-8201) in patients with HER2-expressing metastatic colorectal cancer (DESTINY-CRC01): a multicentre, open-label, phase 2 trial. *Lancet Oncol* 2021; **22**: 779-789 [PMID: 33961795 DOI: 10.1016/S1470-2045(21)00086-3]

155 **Raghav** **KOS**, Yoshino T, Guimbaud R. Trastuzumab deruxtecan in patients with HER2-overexpressing locally advanced, unresectable, or metastatic colorectal cancer (mCRC): A randomized, multicenter, phase 2 study (DESTINY-CRC02). *J Clin Oncol* 2022: **40**: TPS224-TPS224 [DOI: 10.1200/JCO.2022.40.4\_suppl.TPS224]

156 **Moulder SL**, Borges VF, Baetz T, Mcspadden T, Fernetich G, Murthy RK, Chavira R, Guthrie K, Barrett E, Chia SK. Phase I Study of ONT-380, a HER2 Inhibitor, in Patients with HER2(+)-Advanced Solid Tumors, with an Expansion Cohort in HER2(+) Metastatic Breast Cancer (MBC). *Clin Cancer Res* 2017; **23**: 3529-3536 [PMID: 28053022 DOI: 10.1158/1078-0432.CCR-16-1496]

157 **Strickler JH**, Zemla TJ, Ou FS. Trastuzumab and tucatinib for the treatment of HER2 amplified metastatic colorectal cancer (mCRC): Initial results from the MOUNTAINEER trial. *Ann Oncol* 2019

158 **Strickler JH**, Niedzwiecki D, Zemla T. A phase II, open label study of tucatinib (ONT-380) combined with trastuzumab in patients with HER2+ metastatic colorectal cancer (mCRC)(MOUNTAINEER). *J Clin Oncol* 2017; **35**: TPS3624-TPS3624 [DOI: 10.1200/JCO.2017.35.15\_suppl.TPS3624]

159 **Martinelli E**, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP, Normanno N, Rachiglio AM, Maiello E, Latiano T, De Vita F, Ciardiello F. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: challenges and future perspectives. *Ann Oncol* 2020; **31**: 30-40 [PMID: 31912793 DOI: 10.1016/j.annonc.2019.10.007]

160 **Eng C**, Bessudo A, Hart LL, Severtsev A, Gladkov O, Müller L, Kopp MV, Vladimirov V, Langdon R, Kotiv B, Barni S, Hsu C, Bolotin E, von Roemeling R, Schwartz B, Bendell JC. 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**: 177-186 [PMID: 26891420 DOI: 10.1002/ijc.30049]

161 **Cuneo KC**, Mehta RK, Kurapati H, Thomas DG, Lawrence TS, Nyati MK. Enhancing the Radiation Response in KRAS Mutant Colorectal Cancers Using the c-Met Inhibitor Crizotinib. *Transl Oncol* 2019; **12**: 209-216 [PMID: 30412912 DOI: 10.1016/j.tranon.2018.10.005]

162 **Khan KH**, Cunningham D, Werner B, Vlachogiannis G, Spiteri I, Heide T, Mateos JF, Vatsiou A, Lampis A, Damavandi MD, Lote H, Huntingford IS, Hedayat S, Chau I, Tunariu N, Mentrasti G, Trevisani F, Rao S, Anandappa G, Watkins D, Starling N, Thomas J, Peckitt C, Khan N, Rugge M, Begum R, Hezelova B, Bryant A, Jones T, Proszek P, Fassan M, Hahne JC, Hubank M, Braconi C, Sottoriva A, Valeri N. Longitudinal Liquid Biopsy and Mathematical Modeling of Clonal Evolution Forecast Time to Treatment Failure in the PROSPECT-C Phase II Colorectal Cancer Clinical Trial. *Cancer Discov* 2018; **8**: 1270-1285 [PMID: 30166348 DOI: 10.1158/2159-8290.CD-17-0891]

163 **Cremolini C**, Rossini D, Dell'Aquila E, Lonardi S, Conca E, Del Re M, Busico A, Pietrantonio F, Danesi R, Aprile G, Tamburini E, Barone C, Masi G, Pantano F, Pucci F, Corsi DC, Pella N, Bergamo F, Rofi E, Barbara C, Falcone A, Santini D. Rechallenge for Patients With RAS and BRAF Wild-Type Metastatic Colorectal Cancer With Acquired Resistance to First-line Cetuximab and Irinotecan: A Phase 2 Single-Arm Clinical Trial. *JAMA Oncol* 2019; **5**: 343-350 [PMID: 30476968 DOI: 10.1001/jamaoncol.2018.5080]

164 **Martinelli E**, Martini G, Famiglietti V, Troiani T, Napolitano S, Pietrantonio F, Ciardiello D, Terminiello M, Borrelli C, Vitiello PP, De Braud F, Morano F, Avallone A, Normanno N, Nappi A, Maiello E, Latiano T, Falcone A, Cremolini C, Rossini D, Santabarbara G, Pinto C, Santini D, Cardone C, Zanaletti N, Di Liello A, Renato D, Esposito L, Marrone F, Ciardiello F. Cetuximab Rechallenge Plus Avelumab in Pretreated Patients With RAS Wild-type Metastatic Colorectal Cancer: The Phase 2 Single-Arm Clinical CAVE Trial. *JAMA Oncol* 2021; **7**: 1529-1535 [PMID: 34382998 DOI: 10.1001/jamaoncol.2021.2915]

165 **Nakajima H**, Kotani D, Bando H, Kato T, Oki E, Shinozaki E, Sunakawa Y, Yamazaki K, Yuki S, Nakamura Y, Yamanaka T, Yoshino T, Ohta T, Taniguchi H, Kagawa Y. REMARRY and PURSUIT trials: liquid biopsy-guided rechallenge with anti-epidermal growth factor receptor (EGFR) therapy with panitumumab plus irinotecan for patients with plasma RAS wild-type metastatic colorectal cancer. *BMC Cancer* 2021; **21**: 674 [PMID: 34098908 DOI: 10.1186/s12885-021-08395-2]

166 **Hidalgo M**, Martinez-Garcia M, Le Tourneau C, Massard C, Garralda E, Boni V, Taus A, Albanell J, Sablin MP, Alt M, Bahleda R, Varga A, Boetsch C, Franjkovic I, Heil F, Lahr A, Lechner K, Morel A, Nayak T, Rossomanno S, Smart K, Stubenrauch K, Krieter O. First-in-Human Phase I Study of Single-agent Vanucizumab, A First-in-Class Bispecific Anti-Angiopoietin-2/Anti-VEGF-A Antibody, in Adult Patients with Advanced Solid Tumors. *Clin Cancer Res* 2018; **24**: 1536-1545 [PMID: 29217526 DOI: 10.1158/1078-0432.CCR-17-1588]

167 **Xie C**, Zhou J, Guo Z, Diao X, Gao Z, Zhong D, Jiang H, Zhang L, Chen X. Metabolism and bioactivation of famitinib, a novel inhibitor of receptor tyrosine kinase, in cancer patients. *Br J Pharmacol* 2013; **168**: 1687-1706 [PMID: 23126373 DOI: 10.1111/bph.12047]

168 **Xu RH**, Shen L, Wang KM, Wu G, Shi CM, Ding KF, Lin LZ, Wang JW, Xiong JP, Wu CP, Li J, Liu YP, Wang D, Ba Y, Feng JP, Bai YX, Bi JW, Ma LW, Lei J, Yang Q, Yu H. Famitinib versus placebo in the treatment of refractory metastatic colorectal cancer: a multicenter, randomized, double-blinded, placebo-controlled, phase II clinical trial. *Chin J Cancer* 2017; **36**: 97 [PMID: 29273089 DOI: 10.1186/s40880-017-0263-y]

169 **Kuboki Y**, Nishina T, Shinozaki E, Yamazaki K, Shitara K, Okamoto W, Kajiwara T, Matsumoto T, Tsushima T, Mochizuki N, Nomura S, Doi T, Sato A, Ohtsu A, Yoshino T. TAS-102 plus bevacizumab for patients with metastatic colorectal cancer refractory to standard therapies (C-TASK FORCE): an investigator-initiated, open-label, single-arm, multicentre, phase 1/2 study. *Lancet Oncol* 2017; **18**: 1172-1181 [PMID: 28760399 DOI: 10.1016/S1470-2045(17)30425-4]

170 **Webster PJ**, Littlejohns AT, Gaunt HJ, Prasad KR, Beech DJ, Burke DA. AZD1775 induces toxicity through double-stranded DNA breaks independently of chemotherapeutic agents in p53-mutated colorectal cancer cells. *Cell Cycle* 2017; **16**: 2176-2182 [PMID: 28296564 DOI: 10.1080/15384101.2017.1301329]

171 **Hata AN**, Rowley S, Archibald HL, Gomez-Caraballo M, Siddiqui FM, Ji F, Jung J, Light M, Lee JS, Debussche L, Sidhu S, Sadreyev RI, Watters J, Engelman JA. Synergistic activity and heterogeneous acquired resistance of combined MDM2 and MEK inhibition in KRAS mutant cancers. *Oncogene* 2017; **36**: 6581-6591 [PMID: 28783173 DOI: 10.1038/onc.2017.258]

172 **Seligmann JF**, Fisher DJ, Brown LC, Adams RA, Graham J, Quirke P, Richman SD, Butler R, Domingo E, Blake A, Yates E, Braun M, Collinson F, Jones R, Brown E, de Winton E, Humphrey TC, Parmar M, Kaplan R, Wilson RH, Seymour M, Maughan TS; FOCUS4 Trial Investigators. Inhibition of WEE1 Is Effective in TP53- and RAS-Mutant Metastatic Colorectal Cancer: A Randomized Trial (FOCUS4-C) Comparing Adavosertib (AZD1775) With Active Monitoring. *J Clin Oncol* 2021; **39**: 3705-3715 [PMID: 34538072 DOI: 10.1200/JCO.21.01435]

173 **Amatu A**, Sartore-Bianchi A, Siena S. NTRK gene fusions as novel targets of cancer therapy across multiple tumour types. *ESMO Open* 2016; **1**: e000023 [PMID: 27843590 DOI: 10.1136/esmoopen-2015-000023]

174 **Gatalica Z**, Xiu J, Swensen J, Vranic S. Molecular characterization of cancers with NTRK gene fusions. *Mod Pathol* 2019; **32**: 147-153 [PMID: 30171197 DOI: 10.1038/s41379-018-0118-3]

175 **Drilon A**, Laetsch TW, Kummar S, DuBois SG, Lassen UN, Demetri GD, Nathenson M, Doebele RC, Farago AF, Pappo AS, Turpin B, Dowlati A, Brose MS, Mascarenhas L, Federman N, Berlin J, El-Deiry WS, Baik C, Deeken J, Boni V, Nagasubramanian R, Taylor M, Rudzinski ER, Meric-Bernstam F, Sohal DPS, Ma PC, Raez LE, Hechtman JF, Benayed R, Ladanyi M, Tuch BB, Ebata K, Cruickshank S, Ku NC, Cox MC, Hawkins DS, Hong DS, Hyman DM. Efficacy of Larotrectinib in TRK Fusion-Positive Cancers in Adults and Children. *N Engl J Med* 2018; **378**: 731-739 [PMID: 29466156 DOI: 10.1056/NEJMoa1714448]

176 **Vaishnavi A**, Le AT, Doebele RC. TRKing down an old oncogene in a new era of targeted therapy. *Cancer Discov* 2015; **5**: 25-34 [PMID: 25527197 DOI: 10.1158/2159-8290.CD-14-0765]

177 **Cocco E**, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol* 2018; **15**: 731-747 [PMID: 30333516 DOI: 10.1038/s41571-018-0113-0]

178 **Dunn DB**. Larotrectinib and Entrectinib: TRK Inhibitors for the Treatment of Pediatric and Adult Patients With NTRK Gene Fusion. *J Adv Pract Oncol* 2020; **11**: 418-423 [PMID: 33604102 DOI: 10.6004/jadpro.2020.11.4.9]

179 **D'Angelo A**, Sobhani N, Chapman R, Bagby S, Bortoletti C, Traversini M, Ferrari K, Voltolini L, Darlow J, Roviello G. Focus on ROS1-Positive Non-Small Cell Lung Cancer (NSCLC): Crizotinib, Resistance Mechanisms and the Newer Generation of Targeted Therapies. *Cancers (Basel)* 2020; **12** [PMID: 33172113 DOI: 10.3390/cancers12113293]

180 **Doebele RC**, Drilon A, Paz-Ares L, Siena S, Shaw AT, Farago AF, Blakely CM, Seto T, Cho BC, Tosi D, Besse B, Chawla SP, Bazhenova L, Krauss JC, Chae YK, Barve M, Garrido-Laguna I, Liu SV, Conkling P, John T, Fakih M, Sigal D, Loong HH, Buchschacher GL Jr, Garrido P, Nieva J, Steuer C, Overbeck TR, Bowles DW, Fox E, Riehl T, Chow-Maneval E, Simmons B, Cui N, Johnson A, Eng S, Wilson TR, Demetri GD; trial investigators. Entrectinib in patients with advanced or metastatic NTRK fusion-positive solid tumours: integrated analysis of three phase 1-2 trials. *Lancet Oncol* 2020; **21**: 271-282 [PMID: 31838007 DOI: 10.1016/S1470-2045(19)30691-6]

181 **Lev A**, Deihimi S, Shagisultanova E, Xiu J, Lulla AR, Dicker DT, El-Deiry WS. Preclinical rationale for combination of crizotinib with mitomycin C for the treatment of advanced colorectal cancer. *Cancer Biol Ther* 2017; **18**: 694-704 [PMID: 28886275 DOI: 10.1080/15384047.2017.1364323]

182 **Ganesh K**, Stadler ZK, Cercek A, Mendelsohn RB, Shia J, Segal NH, Diaz LA Jr. Immunotherapy in colorectal cancer: rationale, challenges and potential. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 361-375 [PMID: 30886395 DOI: 10.1038/s41575-019-0126-x]

183 **André T**, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, de la Fouchardiere C, Rivera F, Elez E, Bendell J, Le DT, Yoshino T, Van Cutsem E, Yang P, Farooqui MZH, Marinello P, Diaz LA Jr; KEYNOTE-177 Investigators. Pembrolizumab in Microsatellite-Instability-High Advanced Colorectal Cancer. *N Engl J Med* 2020; **383**: 2207-2218 [PMID: 33264544 DOI: 10.1056/NEJMoa2017699]

184 **Diaz LA Jr**, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, Smith D, Garcia-Carbonero R, Benavides M, Gibbs P, de la Fourchardiere C, Rivera F, Elez E, Le DT, Yoshino T, Zhong WY, Fogelman D, Marinello P, Andre T; KEYNOTE-177 Investigators. Pembrolizumab versus chemotherapy for microsatellite instability-high or mismatch repair-deficient metastatic colorectal cancer (KEYNOTE-177): final analysis of a randomised, open-label, phase 3 study. *Lancet Oncol* 2022; **23**: 659-670 [PMID: 35427471 DOI: 10.1016/S1470-2045(22)00197-8]

185 **Overman MJ**, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, Desai J, Hill A, Axelson M, Moss RA, Goldberg MV, Cao ZA, Ledeine JM, Maglinte GA, Kopetz S, André T. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol* 2017; **18**: 1182-1191 [PMID: 28734759 DOI: 10.1016/S1470-2045(17)30422-9]

186 **Morse MA**, Overman MJ, Hartman L, Khoukaz T, Brutcher E, Lenz HJ, Atasoy A, Shangguan T, Zhao H, El-Rayes B. Safety of Nivolumab plus Low-Dose Ipilimumab in Previously Treated Microsatellite Instability-High/Mismatch Repair-Deficient Metastatic Colorectal Cancer. *Oncologist* 2019; **24**: 1453-1461 [PMID: 31147488 DOI: 10.1634/theoncologist.2019-0129]

187 **Yuan J**, Li J, Gao C, Jiang C, Xiang Z, Wu J. Immunotherapies catering to the unmet medical need of cold colorectal cancer. *Front Immunol* 2022; **13**: 1022190 [PMID: 36275766 DOI: 10.3389/fimmu.2022.1022190]

188 **Wu X**, Gu Z, Chen Y, Chen B, Chen W, Weng L, Liu X. Application of PD-1 Blockade in Cancer Immunotherapy. *Comput Struct Biotechnol J* 2019; **17**: 661-674 [PMID: 31205619 DOI: 10.1016/j.csbj.2019.03.006]

189 **Callahan MK**, Odunsi K, Sznol M. Phase 1 study to evaluate the safety and tolerability of MEDI4736 (durvalumab, DUR) + tremelimumab (TRE) in patients with advanced solid tumors. *J of Clin Oncol* 2017; **35**: 3069-3069 [DOI: 10.1200/JCO.2017.35.15\_suppl.3069]

190 **Kanikarla Marie P**, Haymaker C, Parra ER, Kim YU, Lazcano R, Gite S, Lorenzini D, Wistuba II, Tidwell RSS, Song X, Foo WC, Maru DM, Chun YS, Futreal A, Kee B, Menter D, Solis L, Tzeng CW, Parseghian C, Raghav K, Morris V, Chang CC, Jenq R, Tam A, Bernatchez C, Kopetz S, Vauthey JN, Overman MJ. Pilot Clinical Trial of Perioperative Durvalumab and Tremelimumab in the Treatment of Resectable Colorectal Cancer Liver Metastases. *Clin Cancer Res* 2021; **27**: 3039-3049 [PMID: 33811152 DOI: 10.1158/1078-0432.CCR-21-0163]

191 **Vanmeerbeek I**, Sprooten J, De Ruysscher D, Tejpar S, Vandenberghe P, Fucikova J, Spisek R, Zitvogel L, Kroemer G, Galluzzi L, Garg AD. Trial watch: chemotherapy-induced immunogenic cell death in immuno-oncology. *Oncoimmunology* 2020; **9**: 1703449 [PMID: 32002302 DOI: 10.1080/2162402X.2019.1703449]

192 **Fumet JD**, Isambert N, Hervieu A, Zanetta S, Guion JF, Hennequin A, Rederstorff E, Bertaut A, Ghiringhelli F. Phase Ib/II trial evaluating the safety, tolerability and immunological activity of durvalumab (MEDI4736) (anti-PD-L1) plus tremelimumab (anti-CTLA-4) combined with FOLFOX in patients with metastatic colorectal cancer. *ESMO Open* 2018; **3**: e000375 [PMID: 29942666 DOI: 10.1136/esmoopen-2018-000375]

193 **Stein A**, Simnica D, Schultheiß C, Scholz R, Tintelnot J, Gökkurt E, von Wenserski L, Willscher E, Paschold L, Sauer M, Lorenzen S, Riera-Knorrenschild J, Depenbusch R, Ettrich TJ, Dörfel S, Al-Batran SE, Karthaus M, Pelzer U, Waberer L, Hinke A, Bauer M, Massa C, Seliger B, Wickenhauser C, Bokemeyer C, Hegewisch-Becker S, Binder M. PD-L1 targeting and subclonal immune escape mediated by PD-L1 mutations in metastatic colorectal cancer. *J Immunother Cancer* 2021; **9** [PMID: 34315821 DOI: 10.1136/jitc-2021-002844]

194 **Fukuoka S**, Hara H, Takahashi N, Kojima T, Kawazoe A, Asayama M, Yoshii T, Kotani D, Tamura H, Mikamoto Y, Hirano N, Wakabayashi M, Nomura S, Sato A, Kuwata T, Togashi Y, Nishikawa H, Shitara K. Regorafenib Plus Nivolumab in Patients With Advanced Gastric or Colorectal Cancer: An Open-Label, Dose-Escalation, and Dose-Expansion Phase Ib Trial (REGONIVO, EPOC1603). *J Clin Oncol* 2020; **38**: 2053-2061 [PMID: 32343640 DOI: 10.1200/JCO.19.03296]

195 **Formenti SC**, Demaria S. Systemic effects of local radiotherapy. *Lancet Oncol* 2009: **10**: 718-726 [DOI: 10.1016/S1470-2045(09)70082-8]

196 **Segal NH**, Cercek A, Ku G, Wu AJ, Rimner A, Khalil DN, Reidy-Lagunes D, Cuaron J, Yang TJ, Weiser MR, Romesser PB, Stadler ZK, Varghese AM, Ganesh K, Yaeger R, Connell LC, Faleck D, Abou-Alfa GK, Mcauliffe KC, Vaiskauskas P, Solter ML, Ogle M, Adamow MJ, Holland A, Vedantam P, Wong P, Merghoub T, Vakiani E, Hollmann TJ, Juluru K, Chou JF, Capanu M, Erinjeri J, Solomon S, Yamada Y, Kemeny N, Crane CH, Saltz LB. Phase II Single-arm Study of Durvalumab and Tremelimumab with Concurrent Radiotherapy in Patients with Mismatch Repair-proficient Metastatic Colorectal Cancer. *Clin Cancer Res* 2021; **27**: 2200-2208 [PMID: 33504552 DOI: 10.1158/1078-0432.CCR-20-2474]

197 **Zhang C**, Wang Z, Yang Z, Wang M, Li S, Li Y, Zhang R, Xiong Z, Wei Z, Shen J, Luo Y, Zhang Q, Liu L, Qin H, Liu W, Wu F, Chen W, Pan F, Zhang X, Bie P, Liang H, Pecher G, Qian C. Phase I Escalating-Dose Trial of CAR-T Therapy Targeting CEA(+) Metastatic Colorectal Cancers. *Mol Ther* 2017; **25**: 1248-1258 [PMID: 28366766 DOI: 10.1016/j.ymthe.2017.03.010]

198 **Picard E**, Verschoor CP, Ma GW, Pawelec G. Relationships Between Immune Landscapes, Genetic Subtypes and Responses to Immunotherapy in Colorectal Cancer. *Front Immunol* 2020; **11**: 369 [PMID: 32210966 DOI: 10.3389/fimmu.2020.00369]

199 **Kaufman HL**, Lenz HJ, Marshall J, Singh D, Garett C, Cripps C, Moore M, von Mehren M, Dalfen R, Heim WJ, Conry RM, Urba WJ, Benson AB 3rd, Yu M, Caterini J, Kim-Schulze S, Debenedette M, Salha D, Vogel T, Elias I, Berinstein NL. Combination chemotherapy and ALVAC-CEA/B7.1 vaccine in patients with metastatic colorectal cancer. *Clin Cancer Res* 2008; **14**: 4843-4849 [PMID: 18676757 DOI: 10.1158/1078-0432.CCR-08-0276]

200 **Okuno K**, Sugiura F, Hida JI, Tokoro T, Ishimaru E, Sukegawa Y, Ueda K. Phase I clinical trial of a novel peptide vaccine in combination with UFT/LV for metastatic colorectal cancer. *Exp Ther Med* 2011; **2**: 73-79 [PMID: 22977472 DOI: 10.3892/etm.2010.182]

201 **Gatti-Mays ME**, Strauss J, Donahue RN, Palena C, Del Rivero J, Redman JM, Madan RA, Marté JL, Cordes LM, Lamping E, Orpia A, Burmeister A, Wagner E, Pico Navarro C, Heery CR, Schlom J, Gulley JL. A Phase I Dose-Escalation Trial of BN-CV301, a Recombinant Poxviral Vaccine Targeting MUC1 and CEA with Costimulatory Molecules. *Clin Cancer Res* 2019; **25**: 4933-4944 [PMID: 31110074 DOI: 10.1158/1078-0432.CCR-19-0183]

202 **Duan Q**, Zhang H, Zheng J, Zhang L. Turning Cold into Hot: Firing up the Tumor Microenvironment. *Trends Cancer* 2020; **6**: 605-618 [PMID: 32610070 DOI: 10.1016/j.trecan.2020.02.022]

203 **Wu X**, Cai J, Zuo Z, Li J. Collagen facilitates the colorectal cancer stemness and metastasis through an integrin/PI3K/AKT/Snail signaling pathway. *Biomed Pharmacother* 2019; **114**: 108708 [PMID: 30913493 DOI: 10.1016/j.biopha.2019.108708]

204 **Li ZL**, Wang ZJ, Wei GH, Yang Y, Wang XW. Changes in extracellular matrix in different stages of colorectal cancer and their effects on proliferation of cancer cells. *World J Gastrointest Oncol* 2020; **12**: 267-275 [PMID: 32206177 DOI: 10.4251/wjgo.v12.i3.267]

205 **Woolston A**, Khan K, Spain G, Barber LJ, Griffiths B, Gonzalez-Exposito R, Hornsteiner L, Punta M, Patil Y, Newey A, Mansukhani S, Davies MN, Furness A, Sclafani F, Peckitt C, Jiménez M, Kouvelakis K, Ranftl R, Begum R, Rana I, Thomas J, Bryant A, Quezada S, Wotherspoon A, Khan N, Fotiadis N, Marafioti T, Powles T, Lise S, Calvo F, Guettler S, von Loga K, Rao S, Watkins D, Starling N, Chau I, Sadanandam A, Cunningham D, Gerlinger M. Genomic and Transcriptomic Determinants of Therapy Resistance and Immune Landscape Evolution during Anti-EGFR Treatment in Colorectal Cancer. *Cancer Cell* 2019; **36**: 35-50.e9 [PMID: 31287991 DOI: 10.1016/j.ccell.2019.05.013]

206 **Luraghi P**, Reato G, Cipriano E, Sassi F, Orzan F, Bigatto V, De Bacco F, Menietti E, Han M, Rideout WM 3rd, Perera T, Bertotti A, Trusolino L, Comoglio PM, Boccaccio C. MET signaling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. *Cancer Res* 2014; **74**: 1857-1869 [PMID: 24448239 DOI: 10.1158/0008-5472.CAN-13-2340-T]

207 **Hong CS**, Sun EG, Choi JN, Kim DH, Kim JH, Ryu KH, Shim HJ, Hwang JE, Bae WK, Kim HR, Kim KK, Jung C, Chung IJ, Cho SH. Fibroblast growth factor receptor 4 increases epidermal growth factor receptor (EGFR) signaling by inducing amphiregulin expression and attenuates response to EGFR inhibitors in colon cancer. *Cancer Sci* 2020; **111**: 3268-3278 [PMID: 32533590 DOI: 10.1111/cas.14526]

208 **Santos P**, Almeida F. Exosome-Based Vaccines: History, Current State, and Clinical Trials. *Front Immunol* 2021; **12**: 711565 [PMID: 34335627 DOI: 10.3389/fimmu.2021.711565]

209 **Lee SY**, Jeon HM, Ju MK, Kim CH, Yoon G, Han SI, Park HG, Kang HS. Wnt/Snail signaling regulates cytochrome C oxidase and glucose metabolism. *Cancer Res* 2012; **72**: 3607-3617 [PMID: 22637725 DOI: 10.1158/0008-5472.CAN-12-0006]

210 **Taciak B**, Pruszynska I, Kiraga L, Bialasek M, Krol M. Wnt signaling pathway in development and cancer. *J Physiol Pharmacol* 2018; **69** [PMID: 29980141 DOI: 10.26402/jpp.2018.2.07]

211 **Amit S**, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, Mann M, Ben-Neriah Y, Alkalay I. Axin-mediated CKI phosphorylation of beta-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev* 2002; **16**: 1066-1076 [PMID: 12000790 DOI: 10.1101/gad.230302]

212 **He X**, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/beta-catenin signaling: arrows point the way. *Development* 2004; **131**: 1663-1677 [PMID: 15084453 DOI: 10.1242/dev.01117]

213 **Bilic J**, Huang YL, Davidson G, Zimmermann T, Cruciat CM, Bienz M, Niehrs C. Wnt induces LRP6 signalosomes and promotes dishevelled-dependent LRP6 phosphorylation. *Science* 2007; **316**: 1619-1622 [PMID: 17569865 DOI: 10.1126/science.1137065]

214 **Nusse R**, Clevers H. Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. *Cell* 2017; **169**: 985-999 [PMID: 28575679 DOI: 10.1016/j.cell.2017.05.016]

215 **Zarkou V**, Galaras A, Giakountis A, Hatzis P. Crosstalk mechanisms between the WNT signaling pathway and long non-coding RNAs. *Noncoding RNA Res* 2018; **3**: 42-53 [PMID: 30159439 DOI: 10.1016/j.ncrna.2018.04.001]

216 **Kahn M**. Wnt Signaling in Stem Cells and Cancer Stem Cells: A Tale of Two Coactivators. *Prog Mol Biol Transl Sci* 2018; **153**: 209-244 [PMID: 29389517 DOI: 10.1016/bs.pmbts.2017.11.007]

217 **Shoshkes-Carmel M**, Wang YJ, Wangensteen KJ, Tóth B, Kondo A, Massasa EE, Itzkovitz S, Kaestner KH. Subepithelial telocytes are an important source of Wnts that supports intestinal crypts. *Nature* 2018; **557**: 242-246 [PMID: 29720649 DOI: 10.1038/s41586-018-0084-4]

218 **Sebio A**, Kahn M, Lenz HJ. The potential of targeting Wnt/β-catenin in colon cancer. *Expert Opin Ther Targets* 2014; **18**: 611-615 [PMID: 24702624 DOI: 10.1517/14728222.2014.906580]

219 **Fanale D**, Barraco N, Listì A, Bazan V, Russo A. Non-coding RNAs Functioning in Colorectal Cancer Stem Cells. *Adv Exp Med Biol* 2016; **937**: 93-108 [PMID: 27573896 DOI: 10.1007/978-3-319-42059-2\_5]

220 **Trédan O**, Galmarini CM, Patel K, Tannock IF. Drug resistance and the solid tumor microenvironment. *J Natl Cancer Inst* 2007; **99**: 1441-1454 [PMID: 17895480 DOI: 10.1093/jnci/djm135]

221 **Wang MY**, Qiu YH, Cai ML, Zhang CH, Wang XW, Liu H, Chen Y, Zhao WL, Liu JB, Shao RG. Role and molecular mechanism of stem cells in colorectal cancer initiation. *J Drug Target* 2020; **28**: 1-10 [PMID: 31244351 DOI: 10.1080/1061186X.2019.1632317]

222 **Liu X**, Fu Q, Du Y, Yang Y, Cho WC. MicroRNA as Regulators of Cancer Stem Cells and Chemoresistance in Colorectal Cancer. *Curr Cancer Drug Targets* 2016; **16**: 738-754 [PMID: 26577538 DOI: 10.2174/1568009616666151118114759]

223 **Kahn M**. Can we safely target the WNT pathway? *Nat Rev Drug Discov* 2014; **13**: 513-532 [PMID: 24981364 DOI: 10.1038/nrd4233]

224 **Gekas C**, D'Altri T, Aligué R, González J, Espinosa L, Bigas A. β-Catenin is required for T-cell leukemia initiation and MYC transcription downstream of Notch1. *Leukemia* 2016; **30**: 2002-2010 [PMID: 27125305 DOI: 10.1038/leu.2016.106]

225 **Li L**, Peng W, Zhou Q, Wan JP, Wang XT, Qi HB. LRP6 regulates Rab7-mediated autophagy through the Wnt/β-catenin pathway to modulate trophoblast cell migration and invasion. *J Cell Biochem* 2020; **121**: 1599-1609 [PMID: 31544984 DOI: 10.1002/jcb.29394]

226 **Matsuzaki S**, Darcha C. Involvement of the Wnt/β-catenin signaling pathway in the cellular and molecular mechanisms of fibrosis in endometriosis. *PLoS One* 2013; **8**: e76808 [PMID: 24124596 DOI: 10.1371/journal.pone.0076808]

227 **Chen Z**, Venkatesan AM, Dehnhardt CM, Dos Santos O, Delos Santos E, Ayral-Kaloustian S, Chen L, Geng Y, Arndt KT, Lucas J, Chaudhary I, Mansour TS. 2,4-Diamino-quinazolines as inhibitors of beta-catenin/Tcf-4 pathway: Potential treatment for colorectal cancer. *Bioorg Med Chem Lett* 2009; **19**: 4980-4983 [PMID: 19640711 DOI: 10.1016/j.bmcl.2009.07.070]

228 **Chang TS**, Lu CK, Hsieh YY, Wei KL, Chen WM, Tung SY, Wu CS, Chan MWY, Chiang MK. 2,4-Diamino-Quinazoline, a Wnt Signaling Inhibitor, Suppresses Gastric Cancer Progression and Metastasis. *Int J Mol Sci* 2020; **21** [PMID: 32824603 DOI: 10.3390/ijms21165901]

229 **Torres VI**, Godoy JA, Inestrosa NC. Modulating Wnt signaling at the root: Porcupine and Wnt acylation. *Pharmacol Ther* 2019; **198**: 34-45 [PMID: 30790642 DOI: 10.1016/j.pharmthera.2019.02.009]

230 **Wang X**, Moon J, Dodge ME. The development of highly potent inhibitors for porcupine. *J Med Chem* 2013; **56**: 2700-2704 [DOI: 10.1021/jm400159c]

231 **Wang X**, Moon J, Dodge ME, Pan X, Zhang L, Hanson JM, Tuladhar R, Ma Z, Shi H, Williams NS, Amatruda JF, Carroll TJ, Lum L, Chen C. The development of highly potent inhibitors for porcupine. *J Med Chem* 2013; **56**: 2700-2704 [PMID: 23477365 DOI: 10.1021/jm400159c]

232 **Nelson KM**, Dahlin JL, Bisson J, Graham J, Pauli GF, Walters MA. The Essential Medicinal Chemistry of Curcumin. *J Med Chem* 2017; **60**: 1620-1637 [PMID: 28074653 DOI: 10.1021/acs.jmedchem.6b00975]

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

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Target(s)** | **Agent(s)** | **Phase** | **Condition** | **Treatment** | **Main outcomes** |
| EGFR ECD | Sym004 | Phase I | KRAS-WT mCRC | Sym004 | mOS: 12.8 mo |
| EGFR S468R | Necitumumab | Phase II | First-line mCRC | Necitumumab + mFOLFOX6 | ORR: 63.6% |
| KRASG12C | Sotorasib (AMG 510) | Phase I | Solid tumors | Sotorasib | ORR: 7.1%; DCR: 73.8% |
| KRASG12C | 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; T-DM1 | 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-CRC01; phase 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 + FOLFOXIRI | mPFS: 12.1 mo; 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 |
| 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; phase II | dMMR/MSI-H CRC; previously treated | Nivolumab | ORR: 60%; DCR: 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 + FOLFOX | ORR: 81%; DCR: 89% |
| 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 + radiotherapy | ORR: 8.3%; mPFS: 1.8 mo; mOS: 11.4 mo |

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