JBUON 2019; 24(5): 1785-1792
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

REVIEW ARTICLE

# Current and future targets and therapies in metastatic colorectal cancer

Miljana Dzunic[1], Ivan Petkovic[1,2], Ana Cvetanovic[1,2], Svetislav Vrbic[1,2], Ivica Pejcic[1,2]

[1]Clinic of Oncology, Clinical Center Nis, Serbia; [2]Medical Faculty, University of Nis, Serbia.

## *Summary*

*Colorectal cancer (CRC) is the third most common cancer and the second cause of cancer-related deaths worldwide. Despite early diagnosis and treatment improvement, the majority of patients will still suffer from metastatic disease (mCRC), which has a poor prognosis. Molecular diversity of CRC requires personalized targeted approach for improving patient outcomes. Antiangiogenic agents proved to be beneficial in the continuum of mCRC treatment. For efficient epidermal growth factor receptor (EGFR) directed therapy, subtle molecular selection and better strategies to overcome resistance are needed. BRAF mutant and HER-2 positive mCRC will soon be provided with approved targeted treatments and check-point inhibitors demonstrated effectiveness in microsatellite instability (MSI) - high mCRC. Moreover, numeorous promising agents are entering clinical trial arena. This review summarizes actual and possible targets and current and promising agents for mCRC treatment. With broader accessibility of liquid biopsy we could track molecular evolution of CRC and target genetic alterations as they emerge.*

***Key words:*** *metastatic colorectal cancer, precision medicine, targets, treatment*

## Introduction

With more than 1.71 million new cases and 830,000 deaths in 2016, CRC is the third most common cancer and the second cause of cancer-related deaths worldwide [1]. It is estimated that the incidence will increase by 60% in the next decade, especially among young adults, often presented in advanced stage and with aggressive features.

Despite early diagnosis and treatment improvement, up to 75% of patients will still suffer from metastatic disease. Metastatic CRC (mCRC) has a poor prognosis and 5-year overall survival (OS) rate of 13% [2]. The cornerstone of mCRC treatment is cytostatic fluoropyrimidine based therapy in combination with oxaliplatin (FOLFOX) or irinothecan (FOLFIRI). Agents directed towards angiogenesis and EGFR inhibition have brought significant improvement in patient outcomes. Median OS (mOS) for patients with mCRC has been prolonged from 18 months with the use of cytostatic doublets only [6,7] to nearly 30 months by using doublet or triplet (FOLFOXIRI) in combination with targeted therapy [3].

However, the effectiveness of approved targeted therapy is diminished by the lack of predictive markers and development of treatment resistance. Molecular diversity of mCRC requires personalized targeted approach in order to obtain more effective disease control and better survival rates. This review summarizes actual and possible targets as well as current and promising targeted agents for mCRC treatment.

*Corresponding author:* Dzunic, MD. Dr Zoran Djindjic Boulevard 48, 18000 Nis, Serbia
Tel: +381 69 217 16 33, Fax: +381 18 4233 967, E-mail: drmdzunic@gmail.com
Received: 28/10/2018; Accepted: 09/12/2018

## Antiangiogenic treatment

Angiogenesis is a hallmark of CRC progression [9]. Overexpression of vascular endothelial growth factor (VEGF) family of ligands and their interaction with tyrosin kinase receptors (VEGFR-1, VEGFR-2 and VEGFR-3) transfer signals for endothelial cell proliferation, migration and permeability. Bevacizumab (a humanized monoclonal antibody (mAb) that binds all isoforms of VEGF-A) is the only antiangiogenic drug approved for the first line treatment of mCRC [10]. Combined with IFL chemotherapy, bevacizumab improved median progression free survival (mPFS) for 4, 4 months (HR 0.54; p=0.001) and mOS for 4, 7 months (HR, 0.66; p=0.001) compared to IFL, in first line setting [4]. In combination with FOLFOX/XELOX, more modest results were obtained. In second line treatment, bevacizumab and FOLFOX provided survival benefit over FOLFOX, in bevacizumab naïve patients [5]. Several trials demonstrated that keeping bevacizumab beyond progression and changing only chemotherapy backbone in the second line treatment will improve OS, suggesting a benefit of continued anti-VEGF treatment [6].

Aflibercept is a recombinant protein of VEGFR-1 and VEGFR-2 parts fused to IgG1 Fc fragment, which binds VEGF-A, VEGF-B and PIGF. Ramucirumab is a humanized mAb targeting extracellular domain (ECD) of VEGFR-2. Both agents demonstrated superior mOS and mPFS in second line treatment in combination with FOLFIRI compared to FOLFIRI only [7,8], irrespective of prior bevacizumab consumption. Since neither of them was compared directly to bevacizumab, all three are valid antiangiogenic agents for the second line mCRC treatment.

Regorafenib is an oral multikinase inhibitor approved for refractory mCRC, that blocks angiogenesis (VEGFR 1–3, TIE2), oncogenesis (KIT, RET, RAF1, BRAF) and the tumor microenvironment (PDGFR and FGFR). Regorafenib improved mOS from 5.0 months (with placebo) to 6.4 months (HR 0.77; one-sided p=0.0052) in a population of refractory mCRC [18]. Since grade 3-4 toxicity was reported in 50% of patients, research is directed towards finding effective way to mitigate adverse effect of regorafenib. Recently, significant improvement in mOS in chemorefractory KRAS wild type mCRC patients has been reported when regorafenib is given before cetuximab +/- irinothecan, compared to the reverse treatment sequence (HR 0.61, p=0.0293) [10]. A novel oral tyrozin kinase inhibitor (TKI) of VEGFR -1, VEGFR-2 and VEGFR-3, fruquintinib, significantly prolonged mOS (HR 0.65, p<0.001) and mPFS (HR 0.26, p<0.001) compared to placebo in patients with chemorefractory mCRC [11], demonstrated benefit of VEGFR inhibition in the latter lines of mCRC treatment.

## Anti-EGFR therapy

EGFR belongs to the ERBB/HER family of transmembrane receptor tyrosine kinases [21]. Upon activation, it triggers RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR pathway, transferring signals for normal cell growth and survival [22]. Two recombinant mAbs against EGFR, cetuximab (IgG1 human/mouse chimeric) and panitumumab (IgG2κ fully human), are approved for the treatment of mCRC patients without RAS mutations. Cetuximab and panitumumab proved efficacy in combination with chemotherapy regimens FOLFOX [12,13] and FOLFIRI [14,15], in first and second line treatment of mCRC. In chemorefractory patients, cetuximab is used in combination with irinotecan [16,17] or as monotherapy [18] for patients with irinotecan intolerance. Panitumumab proved to be non-inferior to cetuximab as single agent therapy in pretreated patients [30].

## Resistance to anti-EGFR treatment

Cetuximab induced response rate (RR) of only 8-11% of unselected mCRC patients [16,18]. Several biomarkers seem to be predictive of the lack of response to anti-EGFR treatment, inducing primary resistance to anti-EGFR therapy.

Activating mutations in RAS genes are common alterations, presented in around 56% of CRCs. The most frequent is KRAS mutation (40%), predominantly in exon 2, codon 12 and 13. In RAS mutant CRC there is constitutive activation of RAS downstream pathway, promoting tumor proliferation, invasion and dissemination irrespective of EGFR inhibition. In molecularly unselected patients, addition of anti-EGFR mAb to chemotherapy significantly improved RR and PFS, but benefit in OS was lacking. However, in the subgroup of patients without KRAS exon 2 mutation, significant OS improvement was evident, while in patients harboring KRAS exon 2 mutations, no benefit at all, or even harm were reported. Retrospective analyses confirmed the importance of accessing additional RAS mutations (in KRAS exons 3 and 4 and NRAS exons 2, 3 and 4) - "expanded RAS", in order to select patients for anti-EGFR treatment. Finally, meta-analysis showed that patients without RAS mutations had a significantly better treatment outcome with anti-EGFR mAb therapy than RAS mutant patients [20]. RAS mutations are negative predictive markers of cetuximab and panitumumab

activity and patients with known RAS mutation should not be treated with anti-EGFR agents.

Mutations in BRAF, PI3KCA or PTEN loss could be additional biomarkers of primary resistance. Tumors lacking mutations in KRAS, NRAS, BRAF and PIK3CA are named "quadrupole negative" CRCs, having the best chance to respond to anti-EGFR therapy [21]. Moreover, this molecularly selected subgroup could have a benefit of keeping anti-EGFR mAbs beyond progression, and changing only chemotherapy backbone [22]. However, results from randomized phase 3 trials are needed. HER2 and MET amplification/overexpression and hyperproduction of HER3/HER4 ligand herregulin and MET ligand HGF are alterations associated with treatment resistance, however, less frequent [23].

Even if a patient responded to cetuximab/panitumumab, resistance develops in 3-12 months. The most frequent mechanisms of acquired (secondary) resistance are mutations in KRAS/NRAS, emerging in almost 50% of patients. BRAF mutations, activation of IGF-1R pathway, as well as HER2 and MET amplification are also detected. Analyses of circulating tumor DNA (ctDNA) identified mutant allels months before disease progression [24], suggesting that secondary resistance develops mostly by clonal selection of rare preexisted mutated cells under the positive selection pressure from EGFR blockage [49]. EGFR ECD mutation develops de novo and prevents binding anti-EGFR Ab to EGFR. It occurs in about 20% of patients treated with cetuximab, and only 1% treated with panitumumab [25].

## Overcoming resistance to anti-EGFR mAbs

Targeting different epitopes of EGFR could be a strategy to overcome anti-EGFR resistance. Sym004 is a combination of two, and MM-151 of three mAbs to simultaneously engage distinct non-overlapping epitopes on EGFR. In preclinical studies both agents demonstrated profound signal inhibition, stronger antitumor activity than cetuximab and efficacy in tumor cells harboring EGFR ECD mutation. Sym004 was explored versus investigators treatment of choice (capecitabine/ 5-FU/ best supportive care) in chemorefractory KRAS exon 2 wild type mCRC patients with acquired resistance to anti-EGFR therapy. Despite cells with EGFR ECD mutation were efectively targeted, this was not reflected in OS improvement. Subgroup analysis identified patients without RAS/BRAF/ EGFR ECD mutations with OS benefit owing to Sym004 treatment [26]. EGFR ECD point mutation S492R interrupts binding of cetuximab, but does not affect panitumumab binding, suggesting the possibility for clinical evaluation of panitumumab in S492R EGFR ECD mutated patients [27]. Simultaneous tissue and circulating tumor DNA (ctDNA) analysis revealed extensive intra- and interlesional genetic heterogeneity due to cetuximab/panitumumab therapy, which is difficult to target with unique strategy [28]. Accessing early emergence of resistant clones by ctDNA analysis and tailoring the treatment accordingly might be the effective method to reverse resistance to anti-EGFR therapy.

## Targeting RAS mutant CRC

For patients with RAS mutant tumors there are still no specific targeted treatments. Development of drugs targeting activated RAS remains a challenge because of treatment specificity issues. A possible biological strategy might be targeting tumor cells with human reovirus that replicates in RAS mutant cells and induces cell lysis (NCT01274624). The inhibition of mutant RAS might be successful by blocking the pathway at the downstream effector points. In preclinical model a combination of MEK inhibitor trametinib and cyclin dependent kinases (CDK) 4/6 inhibitor palbociclib proved to be well tolerated and highly effective [29]. A combination of PI3K and MEK inhibitor induced tumor regression in mouse model of PI3KCA wild-type, KRAS mutant colorectal cancer [30]. Preclinical studies documented synergistic antiproliferative and apoptotic effects of combined MEK inhibitor or regorafenib with cetuximab [31]. These strategies require further clinical research.

## BRAF mutant CRC

Oncogenic $BRAF^{V600E}$ mutation, as the most common somatic mutation in BRAF gene, is present in approximately 10-15% of CRC patients, causing constitutive, RAS independent activation of BRAF kinase. Patients with $BRAF^{V600E}$ CRC are more frequently females in advanced age, with proximal tumours, and tend to have peritoneal dissemination [32]. BRAF mutation confers poorer prognosis in metastatic and earlier disease settings and may predict a lack of benefit for anti-EGFR mAb therapy, although predictive role has not been well founded [33].

Since BRAF inhibition in $BRAF^{V600E}$ malignant melanoma elicits RR of 60-75% [76], vemurafenib (selective $BRAF^{V600E}$ inhibitor) was evaluated in 21 previously treated BRAF mutant mCRC patients, but derived disappointing RR of 5% [34]. Suggested reason for resistance to BRAF inhibitors in CRC was adaptive feedback signaling via ligand-dependent

EGFR activation, induction of RAS and signaling through other members of RAF family as well as activation of PI3K/AKT pathway [35]. Several clinical studies paired EGFR and BRAF inhibitors. A combination of BRAF inhibitor dabrafenib with MEK inhibitor trametinib yielded RR of 12% and mPFS of 3.5 months [36]. Triple inhibition of EGFR, BRAF and MEK was evaluated by using panitumumab, dabrafenib and trametinib, respectively, and proved to be tolerable and promising therapeutic strategy for $BRAF^{V600E}$ mutant CRC, resulting in ORR of 21%, and mPFS of 4.2 months [37]. A combination of selective RAF inhibitor encorafenib and cetuximab, with PI3Kα inhibitor alpelisib resulted in increased toxicity and no significant therapeutic improvement over doublet (HR 0.69, P=0.064) [38]. Cetuximab and irinotecan proved to be more efficient with the addition of vemurafenib (mPFS 4.4 vs 2.0 months, HR 0.42, p<0.001; RR 16 vs 4%, p=0.09) [39].

The results of the phase 3 trial that was evaluating encorafenib + cetuximab with or without MEK inhibitor binimetinib versus FOLFIRI/irinotecan + cetuximab (BEACON CRC) [40] in progressive BRAF mutant CRC patients have been recently reported. The targeted triple regimen resulted in median OS of 9 months compared to 5.4 months for standard therapy (HR 0.52; 95% CI: 0.39-0.7, p<0.0001), pointing to the novel successful chemotherapy-free targeted regimen for BRAF mutant biology.

Wnt and CDK-inhibitors in combination with BRAF inhibitors and new agents that target both BRAF and C-RAF or ERK are entering the clinical trial arena. A better treatment for BRAF mutant CRC is on the horizon.

## HER-2 amplification/overexpression

HER-2 gene amplification/mutation, leading to excessive PI3KCA/AKT/mTOR signaling, is present in about 5-7% of mCRC patients, predominantly in RAS wild type tumors [41]. In HER2 amplified/overexpressed, heavily pretreated mCRC patients, a combination of trastuzumab (mAb against HER-2 receptor) and lapatinib (TKI targeting EGFR and HER-2) resulted in ORR of 30% and clinical benefit in 74% patients, mPFS was 5 months and mOS 11.5 months [42]. The extent of HER2 amplification/expression correlated with response to treatment. Trastuzumab and pertuzumab (mAb that blocks HER2 dimerization) combination in "HER-2 positive" refractory mCRC produced similar results: ORR 38% and the median duration of response (mDOR) of 11 months [43]. Results suggest that dual anti-HER2 therapy is an effective salvage treatment in pretreated HER-2 overexpressing patients. Phase 3 studies are warranted to obtain more data.

The future research is directed towards positioning anti HER-2 treatment in earlier lines of therapy (NCT03365882), finding the most effective anti HER-2 drug combination (NCT03225937; NCT03043313) and exploring rescue treatment possibilities after progression (NCT03418558). Tucatinib (TKI of HER-2) is assessed in combination with trastuzumab. Neratinib (TKI of EGFR, HER2, and HER4) plus cetuximab will be tested in HER-2 non-amplified, whereas neratinib with trastuzumab will be applied to HER-2 amplified/mutated, quadruple negative mCRC (NCT03457896).

## Immune checkpoint inhibitors

Immunotherapy strategies aim to increase effectiveness of T cells and antitumor immune response by blocking CTLA-4, PD-1 or its ligand PD-L1 with the specific mAbs called checkpoint inhibitors. These agents had poor results in non-selected mCRC patients. However, in patients with dMMR/MSI, checkpoint inhibitors demonstrated an outstanding antitumor effect. MSI is present in about 4-5% of mCRC. It is a characteristic of Lynch syndrome and hereditary CRC [99], but can also be observed sporadically, as a result of biallelic inactivation of MLH1 by promoter hypermethylation [44]. Tumors with dMMR/MSI acquire more somatic mutations compared to MMR proficient tumors [45] and they have increased immunogenicity, manifested by high number of activated tumor infiltrating lymphocytes. Deficient MMR/MSI is predictive for response to immune checkpoint inhibitors and should be assessed in all mCRC patients.

Pembrolizumab (humanized IgG4 MAb against PD1 receptors) in progressive mCRC patients resulted in ORR and PFS rate of 40% and 78%, respectively, for dMMR/MSI-high subpopulation [46]. Similarly, a durable antitumor activity and a manageable safety profile of pembrolizumab was proved in preselected dMMR/MSI-high mCRC patients. Nivolumab (humanized IgG4 MAb directed against PD1) also provided long term responses in 31.1% of pretreated patients with dMMR/MSI-high mCRC [47]. A combination of nivolumab and a low dose ipilimumab (a fully human IgG1 MAb that blocks CTLA-4) demonstrated improved efficacy and a favorable benefit-risk profile in comparison to anti-PD-1 monotherapy, with ORR of 55%, disease control rates (DCR) of 80%, and PFS and OS rates of 71% and 85%, respectively, after 12 months [48]. Based on these results, pembroli-

zumab, nivolumab, and ipilimumab in combination with nivolumab are FDA-approved for the treatment of patients with dMMR/MSI-high mCRC that progressed on chemotherapy. A durable clinical benefit of nivolumab plus low-dose ipilimumab in first line setting has recently been observed and an ongoing phase 3 trial is assessing pembrolizumab versus standard chemotherapy in the first-line setting for dMMR/MSI-high mCRC (NCT02563002). A combination of atezolizumab (humanized IgG1 anti-PD-L1 Ab) and bevacizumab in heavily pretreated MSI-H mCRC patients induced ORR of 30% and DCR of 90% with a tolerable safety profile [49]. Based on preclinical data regarding MEK inhibition enhancing tumor immunogenicity, a combination of atezolizumab and cobimetinib (MEK inhibitor) was evaluated in MSS patients, but failed to prove benefit in PFS and OS over monotherapy with atezolizumab or regorafenib [50]. Numerous strategies to enhance immunogenicity of MSS CRC are being tested to include checkpoint inhibitors in therapeutic armamentarium of larger proportion of mCRC patients.

## Promising future targets

PI3KCA mutations, detected in 10-20% of CRC patients, may coexist with RAS and BRAF mutations and correlate with worse prognosis and resistance to anti-EGFR treatment. Low-dose aspirin seems to improve survival rate in patients with PI3KCA-mutant tumors, by aspirin-mediated COX2 inhibition, which inhibits PI3K signaling. However, this observation requires prospective evaluation [51]. A recent study has reported promising results of the mTOR inhibitor everolimus combined with panitumumab and irinotecan in second line treatment of KRAS wild type mCRC patients [52].

High MET expression is a negative prognostic marker, associated with the treatment resistance. Studies that explored MET inhibition in combination with anti EGFR therapy did not show significant benefit [53]. However, subgroup analysis trended in favor of MET overexpression. Current research is focused on selected mCRC patients with MET gene amplification/overexpression and the use of multikinase inhibitors, such as cabozantinib (activity against MET, VEGFR2, FLT3, c-KIT, and RET) [54].

R–spondine 2 and 3 gene fusions, arising as a result of hromozomal translocations, are presented in 10% of mCRCs, resulting in aberrant Wnt signaling [55]. Inhibition of Wnt pathway can be achieved by using an inhibitor of Porcupine or mAb directed to the fusion protein [56]. However, clinical results have not been established yet. Inactivating mutations of the tumor suppressor genes RNF43 and ZNRF3 define a subset of CRCs dependent on high Wnt levels, where the same strategy can be tested. Preclinical evidence suggests that RET fusions may be effectively targeted with RET – inhibitors, whereas crizotinib (ALK and ROS1 inhibitor) and entrectinib (targets NTRK, ROS1, and ALK) are effective in ALK rearranged CRC [57]. Although with minor frequencies, presence of gene fusions is easily detectable by liquid biopsy, therefore worth conducting further research.

## Conclusion

The studies of targeted treatment in mCRC have revealed a need for assessing a wider range of molecular markers, in order to derive the best possible outcomes for patients. Even though the number of molecularly selected patients with a true benefit from targeted approach is small, efforts are made to broaden its applicability to a larger number of mCRC patients. The treatment of mCRC will change rapidly in the upcoming years, offering hope for patients with worse prognosis. High-sensitivity genome sequencing methods and application of liquid biopsy have provided a deeper insight into not only genetic landscape of mCRC but also molecular evolution and mechanisms of emerging resistance to treatment. Once this technology becomes accessible to everyday clinical practice, we could finally approach the complexity of mCRC and target genetic mutations as they emerge.

## Conflict of interests

The authors declare no conflict of interests.

## References

1. Fitzmaurice C, Akinyemiju TF, Al Lami FH et al. Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-years for 32 Cancer Groups, 1990 to 2016: A Systematic Analysis for the Global Burden of Disease Study. JAMA Oncol 2018;4:1553-68.
2. Siegel RL, Miller KD, Fedewa SA et al. Colorectal cancer statistics. Cancer 2017;67:177-93.

3. Cremolini C, Loupakis F, Antoniotti C et al. 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;16:1306-15.
4. Hurwitz H, Fehrenbacher L, Novotny W et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335-42.
5. Giantonio BJ, Catalano PJ, Meropol NJ et al. Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 2007;25:1539-44.
6. Masi G, Salvatore L, Boni L et al. Continuation or reintroduction of bevacizumab beyond progression to first-line therapy in metastatic colorectal cancer: final results of the randomized BEBYP trial. Ann Oncol 2015;26:724-30.
7. Van Cutsem E, Tabernero J, Lakomy R et al. 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;30:3499-506.
8. Tabernero J, Yoshino T, Cohn AL et al. 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.
9. Grothey A, Van Cutsem E, Sobrero A et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. Lancet 2013;381:303-12.
10. Tsuji Y, Shitara K, Yamanaka T et al. REVERCE: Randomized phase II study of regorafenib followed by cetuximab versus the reverse sequence for metastatic colorectal cancer patients previously treated with fluoropyrimidine, oxaliplatin, and irinotecan–Biomarker analysis. J Clin Oncol 2018;36:557.
11. Li J, Qin S, Xu RH et al. Effect of Fruquintinib vs Placebo on Overall Survival in Patients With Previously Treated Metastatic Colorectal Cancer: The FRESCO Randomized Clinical Trial. JAMA 2018;319:2486-96.
12. Bokemeyer C, Bondarenko I, Hartmann JT et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. Ann Oncol 2011;22:1535-46.
13. Douillard JY, Siena S, Cassidy J et al. 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;28:4697-705.
14. Van Cutsem E, Kohne CH, Hitre E et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 2009;360:1408-17.
15. Peeters M, Price TJ, Cervantes A et al. Randomized phase III study of panitumumab with fluorouracil, leucovorin, and irinotecan (FOLFIRI) compared with FOLFIRI alone as second-line treatment in patients with metastatic colorectal cancer. J Clin Oncol 2010;28:4706-13.
16. Cunningham D, Humblet Y, Siena S et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337-45.
17. Sobrero A, Maurel J, Fehrenbacher L et al. EPIC: Phase III Trial of Cetuximab Plus Irinotecan After Fluoropyrimidine and Oxaliplatin Failure in Patients With Metastatic Colorectal Cancer. J Clin Oncol 2008;26:2311-9.
18. Jonker DJ, O'Callaghan CJ, Karapetis CS et al. Cetuximab for the treatment of colorectal cancer. N Engl J Med 2007;357:2040-8.
19. Price TJ, Peeters M, Kim TW et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. Lancet Oncol 2014;15:569-79.
20. Sorich MJ, Wiese MD, Rowland A, Kichenadasse G, McKinnon RA, Karapetis CS. Extended RAS mutations and anti-EGFR monoclonal antibody survival benefit in metastatic colorectal cancer: a meta-analysis of randomized, controlled trials. Ann Oncol 2015;26:13-21.
21. Bardelli A, Siena S. Molecular mechanisms of resistance to cetuximab and panitumumab in colorectal cancer. J Clin Oncol 2010;28:1254-61.
22. Ciardiello F, Normanno N, Martinelli E et al. Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): a randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX. Ann Oncol 2016;27:1055-61.
23. Bardelli A, Corso S, Bertotti A et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013;3:658-73.
24. Diaz LA, Williams RT, Wu J et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012;486:537-40.
25. Price TJ, Newhall J, Peeters M et al. Prevalence and outcomes of patients (pts) with EGFR S492R ectodomain mutations in ASPECCT: Panitumumab (pmab) vs. cetuximab (cmab) in pts with chemorefractory wild-type KRAS exon 2 metastatic colorectal cancer (mCRC). J Clin Oncol 2015;33:740.
26. Montagut C, Argilés G, Ciardiello F et al. 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.
27. Montagut C, Dalmases A, Bellosillo B et al. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. Nature 2012;18:221-3.
28. Pietrantonio F, Vernieri C, Siravegna G et al. Heterogeneity of Acquired Resistance to Anti-EGFR Monoclonal Antibodies in Patients with Metastatic Colorectal Cancer. Clin Cancer Res 2017;23:2414-22.

29. Ziemke EK, Dosch JS, Maust JD et al. Sensitivity of KRAS Mutant Colorectal Cancers to Combination Therapy That Cotargets MEK and CDK4/6. Clin Cancer Res 2016;22:405-14.
30. Roper J, Sinnamon MJ, Coffee EM et al. Combination PI3K/MEK inhibition promotes tumor apoptosis and regression in PIK3CA wild-type, KRAS mutant colorectal cancer. Cancer Lett 2014;347:204-11.
31. Napolitano S, Martini G, Rinaldi B et al. Primary and Acquired Resistance of Colorectal Cancer to Anti-EGFR Monoclonal Antibody Can Be Overcome by Combined Treatment of Regorafenib with Cetuximab. Clin Cancer Res 2015;21:2975-83.
32. Tran B, Kopetz S, Tie J et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. Cancer 2011;117:4623-32.
33. Rowland A, Dias MM, Wiese MD et al. Meta-analysis of BRAF mutation as a predictive biomarker of benefit from anti-EGFR monoclonal antibody therapy for RAS wild-type metastatic colorectal cancer. Br J Cancer 2015;112:1888-94.
34. Kopetz S, Desai J, Chan E et al. Phase II pilot study of vemurafenib in patients with metastatic BRAF mutated colorectal cancer. J Clin Oncol 2015;33:4032-8.
35. Mao M, Tian F, Mariadason JM et al. Resistance to BRAF inhibition in BRAF-mutant colon cancer can be overcome with PI3K inhibition or demethylating agents. Clin Cancer Res 2013;19:657-67.
36. Corcoran RB, Atreya CE, Falchook GS et al. Combined BRAF and MEK InhibitionWith Dabrafenib and Trametinib in BRAF V600 Mutant Colorectal Cancer. J Clin Oncol 2015;33:4023-31.
37. Corcoran RB, André T, Atreya CE et al. Combined BRAF, EGFR, and MEK Inhibition in Patients with BRAF V600E-Mutant Colorectal Cancer. Cancer Discov 2018;8:428-43.
38. Tabernero J, Geel RV, Guren TK et al. Phase 2 results: encorafenib (ENCO) and cetuximab (CETUX) with or without alpelisib (ALP) in patients with advanced BRAF-mutant colorectal cancer (BRAFm CRC). J Clin Oncol 2016;34:3544.
39. Kopetz S, McDonough S, Morris V et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG 1406). J Clin Oncol 2017;35:520.
40. Huijberts S, Schellens JHM, Fakih M et al. BEACON CRC (binimetinib [BINI], encorafenib [ENCO], and cetuximab [CTX] combined to treat BRAF-mutant metastatic colorectal cancer [mCRC]): A multicenter, randomized, open-label, three-arm phase III study of ENCO plus CTX plus or minus BINI vs irinotecan (IRI)/CTX or infusional 5 fluorouracil/folinic acid/IRI (FOLFIRI)/CTX with a safety lead-in of ENCO + BINI + CTX in patients (Pts) with BRAFV600E mCRC. J Clin Oncol 2017;35 (Abstr TPS3622).
41. Ross JS, Fakih M, Ali SM et al. Targeting HER2 in colorectal cancer: The landscape of amplification and short variant mutations in ERBB2 and ERBB3. Cancer 2018;124:1358-73.
42. Sartore-Bianchi A, Trusolino L, Martino C et al. Dual-targeted therapy with trastuzumab and lapatinib in treatment-refractory, KRAS codon 12/13 wild-type, HER2-positive metastatic colorectal cancer (HERACLES): a proof-of-concept, multicentre, open-label, phase 2 trial. Lancet Oncol 2016;17:738-46.
43. Hainsworth J, Meric-Bernstam F, Swanton C et al. Targeted Therapy for Advanced Solid Tumors on the Basis of Molecular Profiles: Results From MyPathway, an Open-Label, Phase IIa Multiple Basket Study. J Clin Oncol 2018;36:536-42.
44. Veigl ML, Kasturi L, Olechnowicz J et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc Natl Acad Sci USA 1998;95:8698-702.
45. Timmermann B, Kerick M, Roehr C et al. Somatic mutation profiles of MSI and MSS colorectal cancer identified by whole exome next generation sequencing and bioinformatics analysis. PLoS One 2010;5:e15661.
46. Le DT, Uram JN, Wang H et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med 2015;372:2509-20.
47. Overman MJ, McDermott R, Leach JL et al. 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;18:1182-91.
48. Overman MJ, Lonardi S, Wong KYM et al. Durable Clinical Benefit With Nivolumab Plus Ipilimumab in DNA Mismatch Repair-Deficient/Microsatellite Instability-High Metastatic Colorectal Cancer. J Clin Oncol 2018;36:773-9.
49. Hochster HS, Bendell JC, Cleary JM et al. Efficacy and safety of atezolizumab (atezo) and bevacizumab (bev) in a phase Ib study of microsatellite instability (MSI)-high metastatic colorectal cancer (mCRC). J Clin Oncol 2017;35:673.
50. Bendell J, Ciardiello F, Tabernero J et al. LBA-004 Efficacy and safety results from IMblaze370, a randomised Phase III study comparing atezolizumab+cobimetinib and atezolizumab monotherapy vs regorafenib in chemotherapy-refractory metastatic colorectal cancer. Ann Oncol 2018;29:mdy208.003.
51. Liao X, Lochhead P, Nishihara R et al. Aspirin use, tumor PIK3CA mutation, and colorectalcancer survival. N Engl J Med 2012;367:1596-606.
52. Townsend A, Tebbutt N, Karapetis C et al. Phase IB/II Study of Second-Line Therapy with Panitumumab, Irinotecan, and Everolimus (PIE) in KRAS Wild-Type Metastatic Colorectal Cancer. Clin Cancer Res 2018;24:3838-44.
53. Eng C, Bessudo A, Hart LL et al. A randomized, placebo controlled, phase 1/2 study of tivantinib (ARQ 197) in combination with irinotecan and cetuximab in patients with metastatic colorectal cancer with wild-type KRAS who have received first-line systemic therapy. Int J Cancer 2016;139:177-86.
54. Sun Y, Sun L, An Y, Shen X. Cabozantinib, a Novel c-Met Inhibitor, Inhibits Colorectal Cancer Development in a Xenograft Model. Med Sci Monit 2015;21:2316-21.
55. Seshagiri S, Stawiski EW, Durinck S et al. Recurrent R-spondin fusions in colon cancer. Nature 2012;488:660-4.

56. Storm EE, Durinck S, de Sousa e Melo F et al. Targeting PTPRK-RSPO3 colon tumors promotes differentiation and loss of stem-cell function. Nature 2015;529:97-100.
57. Amatu A, Somaschini A, Cerea G et al. Novel CAD-ALK gene rearrangement is drugable by entrectinib in colorectal cancer. Br J Cancer 2015;113:1730-4.