# Precision medicine for metastatic colorectal cancer in clinical practice

Julian E. Riedesser, Matthias P. Ebert and Johannes Betge

*Ther Adv Med Oncol*

2022, Vol. 14: 1–25

DOI: 10.1177/17588359211072703



***Abstract*:** Globally, metastatic colorectal cancer is one of the leading causes for cancer-related death. Treatment limited to conventional chemotherapeutics extended life for only a few months. However, advances in surgical approaches and medical treatment regimens have greatly increased survival, even leading to long-term remission in selected patients. Advances in multiomics analysis of tumors have built a foundation for molecular-targeted therapies. Furthermore, immunotherapies are on the edge of revolutionizing oncological practice. This review summarizes recent advances in the growing toolbox of personalized treatment for patients with metastatic colorectal cancer. We provide an overview of current multimodal therapy and explain novel immunotherapy and targeted therapy approaches in detail. We emphasize clinically relevant therapies, such as inhibitors of MAPK signaling, and give recommendations for clinical practice. Finally, we describe the potential predictive impact of molecular subtypes and provide an outlook on novel concepts, such as functional precision medicine.

***Keywords:*** chemotherapy, colorectal cancer, consensus molecular subtypes, immunotherapy, organoids, patient-derived xenografts, personalized oncology, precision medicine, targeted therapy

Received: 17 August 2021; revised manuscript accepted: 17 December 2021.

Correspondence to:
**Johannes Betge**
Junior Clinical Cooperation Unit Translational Gastrointestinal Oncology and Preclinical Models, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 580, Heidelberg 69120, Germany

DKFZ-Hector Cancer Institute at University Medical Center Mannheim, Mannheim, Germany.

Department of Medicine II, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Mannheim Cancer Center, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany
**j.betge@dkfz.de**

**Julian E. Riedesser**
Junior Clinical Cooperation Unit Translational Gastrointestinal Oncology and Preclinical Models, German Cancer Research Center (DKFZ), Heidelberg, Germany

**Matthias P. Ebert**
Department of Medicine II, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

Mannheim Cancer Center, University Medical Center Mannheim, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

## Introduction

Colorectal cancer is the third most common cancer worldwide and the second leading cause of cancer-related death.[1] In metastatic disease, the prognosis remains poor and most patients cannot be cured.[2] In these patients, chemotherapy is still the mainstay of treatment. However, 5-fluorouracil (5-FU) has been in use since the 1960s and still represents the core of chemotherapy regimens in combination with oxaliplatin[3,4] and irinotecan.[5–7] These chemotherapies are further combined with antibodies against vascular endothelial growth factor (VEGF) signaling (bevacizumab,[8–10] ramucirumab,[11] and aflibercept)[12] or epidermal growth factor (EGF) receptors (cetuximab[13] and panitumumab)[14] in *RAS* wild-type (WT) disease.[15] In addition, trifluridine/tipiracil (TAS-102)[16] and regorafenib[17] are available as third-line/salvage therapy options. These treatments have led to considerable increase in patients' survival to more currently than 30 months.[18] Furthermore, surgical resection has become a standard-of-care option for treating metastases. In fact, metastatic cancer was long believed to necessitate palliative therapy, while today up to 25% of patients with liver metastases have curative potential with 5-year survival of up to 50%.[19] Overall, survival rates have vastly improved with these multimodal concepts and long-term survival is observed in a considerable fraction of patients.[20]

Precision medicine aims to identify the ideal treatment for individual patients by considering the molecular characteristics and specific vulnerabilities of their disease. Different levels of molecular characterization, including immunohistochemical staining, polymerase chain reaction (PCR) tests, next-generation sequencing (panel sequencing, whole exome or whole genome sequencing, RNA-sequencing), and clinical characteristics of the patient, are taken into consideration. To this end,

large-scale sequencing studies have revealed the landscape of molecular alterations present in colorectal cancer within the last decades.[21–24] The most frequent alterations in colorectal cancer, including *APC*, *TP53*, or most *KRAS* mutations, unfortunately cannot be exploited therapeutically yet. Nevertheless, there are several novel medical therapies targeting less frequent molecular alterations, novel immunotherapy strategies, and emerging concepts, such as molecular subtypes and functional precision medicine. In this review, we summarize the current state-of-the-art medical treatment for metastasized colorectal cancer and comprehensively discuss recent advances in precision medicine relevant to this disease, including molecular-targeted therapies and immunotherapies. We provide information on molecular backgrounds of novel therapies and emphasize applications that are relevant for clinical practice.

## Current therapy principles for metastatic colorectal cancer

### *Resection of metastases and chemotherapy*

The general condition of the patient and the ability to tolerate combination chemotherapy, the molecular factors *RAS* (*KRAS*, *NRAS*), *BRAF* and mismatch-repair status (MMR), and the location of the primary tumor (right- vs left-sided) factor into therapy planning.[20] In addition, every patient must be evaluated by a specialist surgeon whether complete resection of all metastases can be achieved.

Patients with resectable metastases of the liver or lung should undergo surgery. Perioperative therapy is usually not performed since data suggest limited or no benefit.[25,26] However, ESMO guidelines suggest preoperative chemotherapy with an oxaliplatin-based regimen (FOLFOX or CAPOX) in patients with unfavorable or unclear prognostic factors, such as synchronous onset of metastases, high number of metastases, suspicion of extrahepatic disease, or high FONG-score.[20,27] In patients with potentially resectable metastases, a conversion therapy is indicated. As there are currently no clear criteria for potentially resectable disease, any patient should principally be considered and regularly reassessed during the course of treatment. Up to 40% of patients with liver metastases become resectable after conversion therapy[28] and survival rates are favorable compared with chemotherapy alone, despite recurrence rates of up to 75%.[20,29] Since response rate is correlated to resection rate,[28] a potent as possible regimen should be used. The exact regimen for this setting is not clearly defined, but usually a chemotherapy doublet (FOLFOX/FOLFIRI) with EGF receptor (EGFR) antibodies is recommended in RAS WT disease[30,31] and a doublet or triplet (FOLFOXIRI) with bevacizumab[32] in RAS mutated cases.[20]

Metastatic colorectal cancer patients with unresectable ('never-resectable') disease are treated in palliative intent with the goals of prolonging survival while keeping good quality of life. Exposure to all active therapeutical substances in combination and in a sequential manner is of importance according to the continuum-of-care concept, which leads to superior survival rates compared with best supportive care.[33] The best possible, that is, most active and best tolerable therapy regimen should be given first. In addition, symptomatic patients may need a more intensive regimen to induce tumor shrinkage, while disease control with a well tolerable therapy is used in never-resectable patients with comorbidities or older patients.[20] Examples for typical treatment courses of colorectal cancer patients with non-resectable metastases are shown in Table 1.

### *Choice of first-line treatment: chemotherapy, RAS status, and primary tumor location*

A chemotherapy doublet with a fluoropyrimidine (5-FU/leucovorin or capecitabine) in combination with irinotecan (FOLFIRI) or oxaliplatin (FOLFOX) is standard of care for most patients. Both FOLFIRI and FOLFOX are equally effective[34,35] but have different side effect profiles. A higher frequency of gastrointestinal toxicity is observed with irinotecan, while peripheral neuropathy is a typical limiting side effect associated with oxaliplatin. 5-FU/leucovorin bolus regimens are more toxic than infusional regimens and have become obsolete.[36] The oral 5-FU prodrug capecitabine can be used instead of i.v. 5-FU/leucovorin in combination with oxaliplatin,[37] but is usually not combined with irinotecan due to higher toxicity than FOLFIRI.[38] Furthermore, EGFR antibodies have not shown a survival benefit with CAPOX (vs CAPOX alone) in the COIN trial and may thus not be used with capecitabine-based regimens.[39] Before starting treatment, dihydropyrimidine dehydrogenase (DPD) polymorphisms can be tested to potentially avoid severe fluoropyrimidine side effects by dose adjustment in affected individuals.[40–42] Testing

**Table 1.** Examples for possible courses of treatment for colorectal cancer patients with non-resectable metastases (adapted from).[20]

| | Example 1 | Example 2 | Example 3 | Example 4 | Example 5 | Example 6 |
|---|---|---|---|---|---|---|
| | RAS WT and left colon | | RAS mutation or right colon | | | Older patient |
| **First line** | FOLFOX + EGFR-Ab | FOLFIRI + EGFR-Ab | CapOx + Bvz | FOLFIRI + Bvz | FOLFOXIRI + Bvz | 5-FU + Bvz |
| **Maintenance** | 5-FU + Bvz | Pause | Cap + Bvz | 5-FU + Bvz | 5-FU + Bvz | Pause |
| **Second line** | FOLFIRI + ramucirumab | CapOx + Bvz | FOLFIRI + aflibercept | FOLFOX + Bvz | FOLFIRI + ramucirumab | FOLFOX (reduced dose) |
| **Third line** | TAS-102 | Irinotecan + EGFR-Ab | TAS-102 | TAS-102 | TAS-102 | |
| **Fourth line** | FOLFOX + EGFR-Ab | TAS-102 | Regorafenib | Regorafenib | | |

has been implemented in several centers (including ours); however, it is to this point controversial according to NCCN[43] or ESMO guidelines.[20] The chemotherapy triplet (FOLFOXIRI) might be more effective than doublet combinations. This may also apply to the combination with bevacizumab. However, data are conflicting and increased toxicity has to be taken into consideration.[44–47] This regimen is therefore only used in selected patients without severe comorbidities and with high need for tumor shrinkage. The composition of chemotherapy (oxaliplatin vs irinotecan, infusional 5-FU vs capecitabine, doublet vs triplet) thus needs to be carefully tailored by the oncologist, taking treatment goals, patients' disease, comorbidities, and preferences into account. In contrast, biological factors determine the choice of molecular antibodies added to chemotherapy. Both VEGF (bevacizumab) and EGFR (cetuximab, panitumumab) antibodies improve outcome when combined with chemotherapy.[8–10,13,14] While there is no predictive biomarker for bevacizumab, EGFR antibodies must only be administered in RAS WT disease.[15] According to data from the FIRE-3 trial, the PEAK trial, and a meta-analysis, treatment with EGFR antibodies and chemotherapy was favorable to bevacizumab with chemotherapy in RAS WT disease.[48–50] In addition, the location of the primary tumor (sidedness) plays a role for the choice of antibody treatment. Tumors located in the right hemicolon (coecum, ascending colon, and transverse colon) are often associated with specific histological and molecular characteristics [low differentiation or medullary morphology, high mucin production, more frequent BRAF mutations and microsatellite instability (MSI)] and a comparably poorer general prognosis.[51] In addition, EGFR antibodies seem to have no benefit in right-sided cancers in first-line therapy. According to retrospective analyses of clinical trials,[52,53] cetuximab with FOLFIRI was superior to FOLFIRI alone in left-sided disease (HR 0.65) but not in right-sided cases (HR 1.08).[52] FOLFIRI with cetuximab had longer overall survival (OS, 38.3 months) than FOLFIRI with bevacizumab (28 months) in left-sided RAS WT disease. In comparison, right-sided tumors had generally shorter OS with cetuximab in combination with FOLFIRI (18.3 months) and worse outcome than with bevacizumab (23 months, not significant).[52] Hence, bevacizumab can be used in first line in all patients independent of RAS and sidedness, but cetuximab or panitumumab is preferred in RAS WT cancers originating from the distal colon.

### *Maintenance therapy*

After first-line induction therapy, patients who respond to treatment but cannot undergo resection are usually switched to maintenance therapy after 4–6 months because of the toxicity of combination regimens. This is of special importance in case of oxaliplatin-based therapies, due to peripheral neuropathy. Maintenance strategies after induction therapy with oxaliplatin-based regimens have consequently been investigated in several trials. It was shown that a less toxic maintenance treatment with a fluoropyrimidine together with bevacizumab led to favorable progression-free survival (PFS) compared with drug holidays and to similar PFS compared with

continuous treatment. The data on OS were less clear, trending toward an advantage of maintenance therapy versus drug holidays.[54–58] However, at least according to one meta-analysis, no clinically significant reduction of OS was reported for intermittent versus continuous treatment strategies.[58] Hence, maintenance therapy is generally recommended for most patients, but treatment discontinuation may be discussed with the patient in selected cases.

### *Later-line treatments and therapy sequence*

A second-line treatment is generally recommended for most patients, as it has been associated with prolonged survival.[59] Therapeutic strategies are based on patient- and disease-related factors, and the previous therapy (reviewed in detail by Giordano et al.[60]). Regarding the chemotherapy backbone, usually a switch is recommended: patients who received irinotecan (+5-FU) switch to oxaliplatin (and fluoropyrimidine) and patients who received oxaliplatin (and fluoropyrimidine) switch to irinotecan together with 5-FU.[34] Irinotecan (without 5-FU) may also be given as chemotherapy in second- or later-line treatments if appropriate. With respect to antibody therapy, patients having received anti-EGFR antibodies in first line usually switch to anti-angiogenesis in second line. Bevacizumab has been shown to be effective in second line in combination with chemotherapy and can be given beyond progression in first line.[61–63] Other anti-angiogenic strategies in second line include aflibercept[12] or ramucirumab[11] in combination with FOLFIRI. Anti-EGFR antibodies have also shown activity in second line in *RAS* WT disease, both as single agents and together with chemotherapy,[64,65] but are not continued after treatment failure in first line. As mentioned above, the sequence EGFR antibody (first line) followed by VEGF(R) antibody (second line) seems to be favorable over VEGF➔EGFR in RAS WT disease[66,67] Sidedness may also be predictive for EGFR antibody response in second and later therapy lines,[68,69] but evidence is currently less definte,[70] so that cetuximab or panitumumab may be given in right-sided cases in later treatment lines at this point.[43]

'Last line' therapy options include nucleoside-analog TAS-102[16] and multi-tyrosine kinase inhibitor regorafenib.[17] Combination therapies of TAS-102 with anti-angiogenic antibodies have shown promising results in phase II clinical trials,[71,72] and combinations are evaluated also in earlier treatment lines. TAS-102, alone or in combination with bevacizumab, is therefore a recommended treatment option in patients who have progressed beyond standard therapies.[43] Beyond or alternatively, rechallenge and reintroduction remain frequently used options in the later-line setting of patients with good performance status. Reintroduction is the administration of a former treatment regimen the patient benefited from, but that was terminated (mostly due to toxicities), while rechallenge is the readministration of a treatment regimen toward the patient has previously developed a resistance.[73] Data supporting these concepts are, however, limited. Further options, including immunotherapies or targeted therapies based on molecular characterization and molecular tumor boards, have become available recently for selected patients. These will be described in the following paragraphs.

## MSI and checkpoint inhibitors

Targeting the immune system has become the third mainstay of medical oncology next to chemotherapy and targeted therapies. Clinically approved concepts are based on antibodies directed against immune checkpoints, such as the programmed cell death 1 (PD-1), its ligand (PD-L1), or cytotoxic T-lymphocyte-associated protein 4 (CTLA4). These checkpoints transduce inhibitory signals to T cells leading to immune evasion of cancers, while antibodies directed against them conversely disinhibit T cell function leading to cancer cell killing.[74] Response to immune checkpoint antibodies has been hypothesized to be associated with the number of mutations present in cancers, the latter leading to expression of neo-epitopes increasing the cancers' visibility for the immune system.[75] A high tumor mutational burden (TMB) is also associated with T cell infiltration and PD-L1 expression in tumors.[76] The majority of colorectal cancers harbor only low to moderate numbers of mutations; however, there is a hypermutated subgroup of colorectal cancer encompassing roughly 5% of metastatic tumors.[77] The majority of these hypermutated tumors are characterized by defects in the DNA mismatch-repair (MMR) system leading to MSI.[22] Le et al.[78] recruited patients with colorectal cancers and other tumors with MMR deficiency (dMMR) and MMR proficient (pMMR) colorectal cancers in a phase II study to test clinical activity of the checkpoint inhibitor

pembrolizumab. In this seminal study, they found an impressive 40% response rate and 78% 20-week PFS rates for dMMR tumors (vs 0% and 11% in pMMR colorectal cancers). After further studies in colorectal and non-colorectal dMMR tumors confirming durable response with this therapy, the Food and Drug Administration (FDA) issued morphology-agnostic approval of pembrolizumab in dMMR cancers in 2017.[79–82] In addition, nivolumab, another PD-1 antibody, has shown durable responses (response rate 31%, disease control 69%) and 73% 12-month OS in pretreated dMMR colorectal cancer patients according to the phase II CheckMate 142 trial.[83] The combination of nivolumab with CTLA4 antibody ipilimumab further increased efficacy (response rate 55%, disease control rate 80%, 12-month OS 85%) in another arm of the same trial.[84] These results prompted FDA approval of nivolumab (2017) and nivolumab/ipilimumab (2018) for pretreated dMMR metastatic colorectal cancer. Other checkpoint inhibitors, including PD-L1 antibodies atezolizumab, durvalumab, and avelumab, have also been studied in early clinical trials and have shown encouraging results in dMMR colorectal cancer.[85–87] Checkpoint inhibitors have been investigated in the first-line setting of metastatic dMMR/MSI colorectal cancer. The combination of nivolumab and ipilimumab showed 24-month PFS and OS of 74% and 79%, respectively, while median PFS and OS were not yet reached after median follow-up of 29 months.[88] Pembrolizumab was tested in the first-line setting against investigators' choice combination chemotherapy (FOLFOX/FOLFORI ± bevacizumab or cetuximab) in the Keynote-177 phase III trial (NCT02563002).[89] In the updated analysis, the pembrolizumab group had a lower risk of death, although significance was not reached (HR 0.74). Median PFS was 16.5 months in the pembrolizumab group versus 8.2 months in the chemotherapy group, while the overall response rate (ORR) was also higher in the pembrolizumab group (45.1% vs 33.1%). In addition, adverse events were significantly lower in the pembrolizumab group.[90] Hence, checkpoint inhibitor therapy is becoming the standard of care in first-line setting of dMMR colorectal cancer and dMMR/MSI testing must be done in every colorectal cancer patient.

### *Immunotherapy for non-dMMR cancers?*

While immunotherapy is becoming standard of care in dMMR colorectal cancer, the majority (approx. 95%) of metastatic colorectal cancers are characterized by pMMR/microsatellite stable (MSS) status. In these patients, efficacy of immunotherapy as monotherapy has been disappointing. In addition, not all patients with dMMR disease respond to checkpoint inhibitors. Therefore, current studies aim to identify predictive markers to improve patient stratification and to establish combination strategies for improving efficacy, especially in patients with pMMR tumors. Several studies are testing combination strategies of checkpoint inhibitors with chemotherapy, radiation, anti-VEGF antibodies, anti-EGFR antibodies, inhibitors of mitogen-activated protein kinase (MAPK) signaling, or multi-tyrosine kinase inhibitors aiming to turn immunologically 'cold' tumors into 'hot' tumors and thereby making them susceptible to immunotherapies (reviewed in Pecci et al.[91] and Hirano et al.,[80] Figure 1).

According to preliminary results, combinations of checkpoint inhibitors with VEGF antibodies or multi-tyrosine kinase inhibitors (also targeting VEGF receptors) appear promising. Preclinical studies in different tumor types have reported on endothelial-mediated (or VEGF-mediated) immunosuppression within tumors, which may be overcome with this dual strategy.[92,93] In addition, targeting VEGF may improve T cell infiltration into the tumor microenvironment.[94,95] Accordingly, combination strategies have been successful in hepatocellular carcinoma, renal cell cancer, or lung cancer, also in combination with chemotherapy.[96–98] In colorectal cancer, the BACCI phase II trial assessed atezolizumab in combination with bevacizumab and capecitabine versus placebo with bevacizumab and capecitabine in 133 pretreated metastatic colorectal cancer patients.[99] The combination showed a modest (but statistically significant) benefit in PFS of 4.4 months versus 3.3 months, thereby reaching its prespecified primary end point. The phase I REGONIVO trial assessed regorafenib with nivolumab in 50 pretreated patients with colorectal or gastric cancers (25 patients each). In the colorectal cancer cohort, 36% objective response and a PFS of 7.9 months was observed with a manageable safety profile. This was considered an encouraging result warranting further study in larger trials.[100] In contrast, the REGOMUNE trial, testing regorafenib and avelumab in pMMR colorectal cancers reported no objective responses (stable disease in 54% of 48 patients), but the authors noted recruitment of antitumor immunity in a subset of

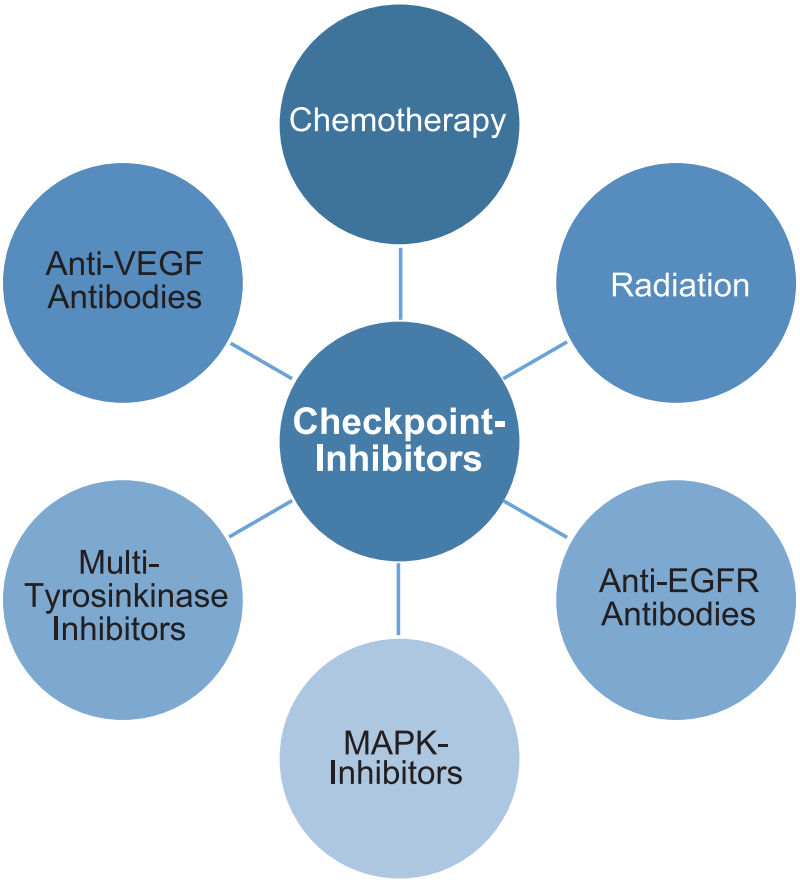


**Figure 1.** Combination strategies to enhance immune checkpoint inhibitor efficacy in pMMR colorectal cancers tested in current clinical trials.

patients, supporting the general concept.[101] Currently ongoing studies also test checkpoint inhibitors together with bevacizumab and chemotherapy in first-line setting. The NIVACOR[102] and ATEZOTRIBE (NCT03721653) trials, for instance, investigate the combination of intensified chemotherapy with FOLFOXIRI together with bevacizumab and checkpoint inhibition (nivolumab or atezolizumab, respectively). According to preliminary results from NIVACOR, the combination was generally well tolerated, and efficacy data are pending.

The importance of MAPK signaling in colorectal cancer therapy is discussed in detail below. With respect to immunotherapy, the pathway has been implicated in tumor–immune interaction in multiple ways, including regulation of immunosuppression through cytokines and growth factors, regulation of human leukocyte antigen (HLA) expression and thereby immune cell evasion from the tumor microenvironment.[91] The combination of atezolizumab with MEK inhibition using cobimetinib, however, led to disappointing efficacy in the phase III IMblaze370 trial, analyzing 363 pretreated patients: the combination led to an OS of 8.9 months compared with 7.1 months for atezolizumab alone and 8.5 months with the control treatment regorafenib.[103] Clinical trials combining checkpoint inhibitors with EGFR antibodies cetuximab and panitumumab with and without chemotherapy are ongoing.[80] Cetuximab has previously been shown to induce antibody-dependent cell-mediated cytotoxicity, which could synergize with checkpoint inhibitors.[104] For instance, the AVETUX phase II trial tested the combination of avelumab and cetuximab together with FOLFOX in the first-line setting. Overall, 39 patients of the intention-to-treat (ITT) cohort reached a 79.5% ORR and 92.3% disease control rate, thus a randomized trial appears feasible.[105]

In summary, while immune checkpoint inhibitor therapy has become standard of care in dMMR/

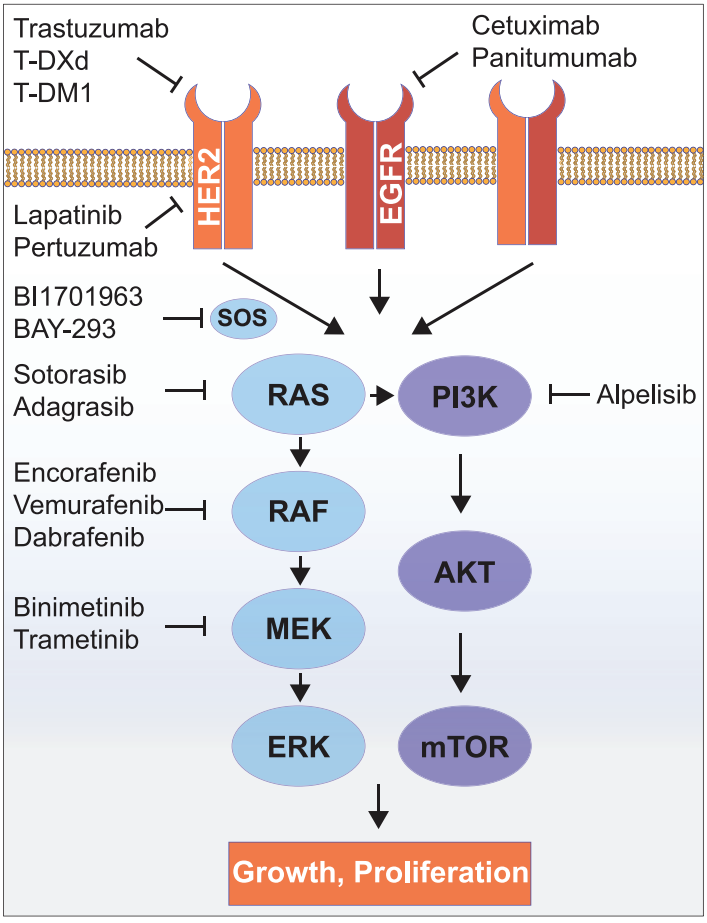


**Figure 2.** Targeting the MAPK pathway in colorectal cancer. Druggable receptors and intracellular signaling components of the pathway are depicted. Drugs targeting the pathway discussed in the text are highlighted. T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan.

MSI colorectal cancer, finding combination therapies for enhancing efficacy in dMMR cases remains challenging. Results from larger studies pertaining this group are awaited in the next months and years. Higher-order combinations of checkpoint inhibitors with targeted therapies and chemotherapeutics increase the chance of efficacy by elevating the probability that combinations include an effective drug.[106] This strategy, however, may lead to enhanced side effects, demanding for more extensive clinical and preclinical biomarker discovery for better stratification of patients.

## Targeting the MAPK pathway

The EGF/MAPK pathway is an intercellular and intracellular signal transduction cascade that regulates a plethora of processes, most importantly not only proliferation, cell growth, and apoptosis but also metabolism or migration of cells and others (Figure 2). The ligands, such as EGF, bind to the human EGF 1–4 (HER1–4 or ERBB1–4) family of transmembrane receptors. HER1 is also referred to as EGFR. Ligand binding leads to dimerization of the receptor, either as homo- or heterodimer (e.g. HER1 with HER2), followed by a cross-phosphorylation of the intracellular receptor domains. Prompted by this phosphorylation, Src homology and collagen (SHC) binds to the receptor and associates growth factor receptor binding protein 2 (GRB2) that in turn recruits son of sevenless (SOS) from the cytosol.[107–109] SOS is a guanine exchange factor that catalyzes the exchange from RAS-GDP to RAS-GTP, thereby activating the RAS protein.[110–112] Activated RAS-GTP in turn directly activates downstream components of the MAPK pathway, for example, RAF, PI3K, and several other effectors.[113] The rapidly accelerated fibrosarcoma (RAF) protein family includes BRAF[114] and is activated by RAS-GTP through direct

interaction.[115] Active RAF phosphorylates and thereby activates the dual-specificity protein kinases MEK1 and MEK2.[116] They again phosphorylate and activate their substrates ERK1 and ERK2 (extracellular signal-regulated kinase), which phosphorylate and activate several cytosolic and nuclear substrates, activating transcription of proteins that enhance and promote proliferation, growth, evasion of apoptosis and affecting various cellular functions, including metabolism, migration, angiogenesis, and immune regulation.[117] Based on these physiological functions, the MAPK pathway plays a prominent role in the development of cancer.

In colorectal cancer, MAPK pathway alterations are very common (59% of non-hypermutated and 80% of hypermutated cases) and most prominently affect *KRAS*, *NRAS*, or *BRAF*.[22] Several antibodies and small molecule inhibitors of the pathway have been developed that are being tested in clinical trials or entered clinical use (Figure 2). As previously discussed, EGFR antibodies, cetuximab and panitumumab, are included in standard-of-care first-line therapy regimens for *RAS* WT, left-sided colorectal cancer. Small molecules targeting EGFR are also available, but do not play a role in clinical use as they were found to be less effective while having enhanced toxicities in colorectal cancer. In the next paragraph, we will highlight the most important developments for clinical management of cancers with alterations in *HER2*, *BRAF*, and *KRAS*, for which direct targeting has become a clinically meaningful option.

### *HER2-targeted therapy*

The HER2 (ERBB2) receptor is similar to EGFR in its structure and function, inducing downstream signaling particularly through MEK/ERK and PI3K/AKT, which leads (among other functions) to mitogenic stimuli, cell growth, and survival.[118] Inhibiting this tumorigenic function with antibodies and small molecules has become a standard treatment in breast cancer and gastric cancer. Its broad activation in several other cancers, including that of the colorectum, has also made it an emerging treatment option for these diseases.[119] The activation of HER2 signaling within the oncogenic process depends on overexpression due to gene amplification in the majority of cases.[120,121] Specifically, this leads to the formation of homodimers or heterodimers of the receptor with other HER family members and subsequent activation of downstream pathways.[122] While several mutations in *HER2* have been found in large-scale sequencing projects,[123] not all of them seem to be activating.[119,120,124] In histopathological assessment, usually a strong expression (3+) in immunohistochemistry (IHC) or a 2 + expression together with a detection of amplification in *in situ* hybridization (ISH) is considered as HER2-high and predictive of response to HER2 therapies. The association of lower HER2 expression levels (2 + expression in IHC without amplification in ISH or 1 + expression) or *HER2* mutations with treatment response is less clear.[125] Trastuzumab was the first HER2 antibody clinically approved after showing significantly improved OS in combination with chemotherapy in HER2 positive breast cancer and has since then emerged as a standard-of-care in this disease.[126] The results of the TOGA trial[127] also led to approval of trastuzumab in combination with chemotherapy in HER2 positive metastatic gastric and gastroesophageal junction cancers. Other drugs targeting HER2, which are approved in breast cancer and are being studied in other solid tumors, include pertuzumab (an antibody with a different binding site than trastuzumab), and small molecules lapatinib (targeting both HER2 and EGFR) and neratinib (inhibitor of HER1, 2, and 4).[119,120] While the frequency of HER2 overexpression is reported to be 20–25% in breast cancer and 10–15% in gastroesophageal adenocarcinoma, only about 3–5% of colorectal cancers harbor HER2 amplifications.[119,128] Nevertheless, effective targeting of HER2 in breast and gastroesophageal cancer has led to efforts to determine if it is exploitable as a target in colorectal cancer.[129] Interestingly, *RAS* WT HER2 overexpressing colorectal cancer has been associated with poor prognosis and resistance toward EGFR antibody treatment.[130] To this end, mouse xenograft studies suggested a compensatory upregulation of HER2 on EGFR antibody treatment, revealing an acquired resistance mechanism toward EGFR-targeted therapy through HER2.[131] This suggested a potential salvage therapy option for advanced colorectal cancer patients refractory to EGFR antibodies. Accordingly, the HERACLES phase II trial tested a combination of trastuzumab and lapatinib in HER2 positive metastatic colorectal cancer refractory to standard of care (including cetuximab or panitumumab).[132] However, 27 of 914 screened patients were eligible for trial and 8 of those (30%) had an objective response, while 12 more patients (44%) had stable disease (disease control rate 74%). Median

PFS was 5.2 months, OS was 11.5 months, and the treatment was well tolerated in this heavily pretreated group (median of five previous therapy lines). Consequently, these results were rightfully interpreted as very promising. Further substantiating these results, the subgroup of colorectal cancers with HER2 activation (amplification, overexpression, or activating mutation) included in the MyPathway basket trial also showed a notable objective response rate of 38% (14 of 37 patients) and a PFS of 5.6 months.[133] More recently, HER2 antibody drug conjugates, such as trastuzumab emtansine[134,135] or trastuzumab deruxtecan,[136] have been introduced. Early basket clinical trials for different pretreated tumor entities, including colorectal cancer, had promising results.[137,138] In colorectal cancer, however, the HERACLES-B trial, testing trastuzumab emtansine with pertuzumab in 31 heavily pretreated patients, did not reach the predefined efficacy end point of ORR (9.7%, estimated 30%), but led to stable disease in 67.7% of patients and a PFS of 4.1 months. Nausea and fatigue were the most frequent adverse events. Most recently, data from the phase II DESTINY-CRC01 trial, evaluating trastuzumab deruxtecan in pretreated metastatic colorectal cancer patients, were published.[139] Overall, 78 patients were enrolled in this study, 58 of those were with HER2 3 + or 2 + and ISH positive cancers. In these patients with the highest HER2 expression status, an objective response was observed in 24 patients (45.3%). Neutropenia and anemia were the most frequent adverse events, interstitial lung disease, or pneumonitis occurred in 5% of patients. Thus, especially trastuzumab deruxtecan appears to be a promising agent for HER2 amplified colorectal cancer. It will be interesting to see more survival and quality of life data with this drug soon. In addition, further studies should test the impact of different HER2 mutations on efficacy of HER2-targeted therapy.

We can conclude that targeting of HER2 in RAS-WT colorectal cancer refractory to standard therapy is a viable treatment option on basis of the HERACLES and the MyPathway trial results. We therefore recommend HER2 analysis in a fresh tumor biopsy and subsequent treatment of HER2 positive patients, ideally within a clinical trial.

### *Treatment of BRAF mutant cancers*

*BRAF* mutations can be found in 5–10% of colorectal cancers, predominantly the V600E type.[140] This mutation increases the catalytic activity of BRAF and reduces autoinhibition, thereby leading to a constitutive activation of the MAPK pathway.[141] The *BRAF* V600E mutation is associated with poor prognosis and generally a poor response to therapy.[142] Further typical features of *BRAF* V600E-mutated colorectal cancers are right-sided tumor location, poor tumor differentiation, peritoneal metastases,[143,144] and hypermethylation, MSI, and CMS subtype 1 (compare below).[145] Surprisingly, *BRAF* mutations other than V600E might be associated with better survival.[146]

Due to the association with poor response and prognosis, treatment of *BRAF* V600E-mutated colorectal cancer is challenging. Resection of metastases is controversially discussed in this subgroup and should only be performed after careful patient selection.[147,148] An intense regimen is often recommended for fit patients in first line, usually a cytotoxic triplet (FOLFOXIRI) in combination with bevacizumab.[20] This recommendation is mainly based on the results of the phase III TRIBE trial comparing an irinotecan-based cytotoxic doublet (FOLFIRI) plus bevacizumab to a triplet (FOLFOXIRI) plus bevacizumab. In a subgroup analysis of 28 BRAF V600E cases, both OS (19.0 vs 10.7 months) and PFS (7.5 vs 5.5 months), were better in the FOLFOXIRI arm. Significance was not reached in both cases, potentially due to the small sample size.[47] In the following phase III TRIBE-2 study, the experimental group received FOLFOXIRI plus bevacizumab as first-line induction therapy, while the control arm received FOLFOX plus bevacizumab. Maintenance treatment consisted of 5-FU plus bevacizumab in both groups. After progression, the experimental group received FOLFOXIRI plus bevacizumab again, while the control group was treated with FOLFIRI plus bevacizumab. In a post hoc subgroup analysis, the strong benefit from the TRIBE study could not be confirmed, which was explained by the different treatment regimen in the control group.[149] In an individual patient data, meta-analysis of five trials comparing FOLFOXIRI plus bevacizumab with doublet plus bevacizumab, no benefit for the triplet in the *BRAF-mutated* subgroup was observed. This was assumed to be caused by the different doublet therapy regimen in TRIBE (FOLFIRI) versus all other trials (FOLFOX). It can be concluded that FOLFORIXI plus bevacizumab should no longer be the first choice for *BRAF* V600E-mutated patients, but FOLFOX plus bevacizumab indeed seems to be preferrable.[150]

In second line, due to the association of *BRAF* mutations with MSI, some patients benefit from immune checkpoint inhibitors, so that MMR and MSI should always be tested.[89] Targeted therapy has become another option for the remaining majority of patients recently. Over the last decade, several BRAF-inhibitors have been established, including vemurafenib, dabrafenib, or encorafenib. These inhibitors showed encouraging response rates in advanced BRAF V600E-mutated non-small-cell lung cancers[151–153] and improved survival of patients with advanced melanoma bearing a *BRAF* V600E mutation.[154–156] Attempts to target *BRAF* V600E in metastatic colorectal cancer as monotherapy have been largely unsuccessful, though. In a phase I dose expansion study with encorafenib, about two-thirds of 18 patients showed stable disease but no patient responded.[157] In a phase II study of 21 patients treated with vemurafenib, 1 patient had a partial response, while one-third of patients had a stable disease.[158] Similar results were obtained in a basket trial of vemurafenib treatment in BRAF-mutated non-melanoma cancers: no objective response was detected in 10 colorectal cancer patients, 50% had stable disease. These rather disappointing results can be explained by a feedback activation of EGFR as response to BRAF V600E inhibition, which, however, could be overcome by EGFR inhibition in preclinical models.[159,160] This combination of vemurafenib and cetuximab was also tested in the basket trial mentioned above. An increase in response rate to 4% and disease control rate to 73% was noted.[151] Further drugs have been added to the combination of BRAF and EGFR inhibition in clinical trials to improve efficacy. The cytotoxic agent irinotecan was tested in combination with cetuximab and vemurafenib in a phase I study leading to a response rate of 35% and stable disease in 53% of patients.[161] The combination of encorafenib and cetuximab was tested with and without the PI3K inhibitor alpelisib in another phase I study. ORR was 19.2% in the group without alpelisib and 17.9% with alpelisib, while stable disease was noted in 57.7% and 75.0%, respectively, leading to a disease control rate of 76.9% versus 92.9%.[162] Another study compared different combinations of the MEK inhibitor trametinib (T), the EGFR antibody panitumumab (P), and the BRAF inhibitor dabrafenib (D). Response rates were 0% for the T + P group, 10% for the D + P group, and 21% for the triple combination of T + P + D, underlining the stronger effect of combinations of inhibitors of the MAPK pathway.[163]

Based on the promising results of double and triple combinations, the open-label, randomized phase III BEACON trial recruited 665 patients with *BRAF* V600E metastatic colorectal cancer that had previously received one or two therapy regimens.[164] Patients received either a triple combination of encorafenib (BRAF), cetuximab (EGFR), and binimetinib (MEK), or a doublet combination of encorafenib and cetuximab. The control group received either irinotecan or FOLFIRI combined with cetuximab according to investigators' choice. Median OS (primary end point) was 9.0 months within the triplet group and 8.4 months in the doublet group versus 5.4 months in the control group. Compatible results were obtained regarding the response rates: objective response rate was 26% for the triplet group, 20% for the doublet group, and 2% for the control group. Interestingly, the experimental arms were also superior regarding adverse events of grade 3 or higher: 58% triplet, 50% doublet, and 61% control. These results led to FDA and European Medicines Agency (EMA) approval for the doublet combination of encorafenib and cetuximab as second-line treatment regimen for metastatic *BRAF* V600E-mutated colorectal cancer, thereby changing current clinical practice. Based on the results of the BEACON trial, the single-armed phase II ANCHOR trial aims to explore encorafenib, binimetinib, and cetuximab as a first-line therapy regimen. During the phase I stage of this trial, an objective response rate of 50% and a median PFS of 4.9 months could be observed, so that phase II within this trial was initiated and results are expected soon.[165] The observed PFS is in the range of current standard therapy FOLFOXIRI + bevacizumab.[47] If efficacy and safety prove to be encouraging, a phase III study will be needed to establish the targeted regimen as first-line treatment. The phase III BREAKWATER (NCT04607421) trial is currently evaluating encorafenib and cetuximab ± cytotoxic chemotherapy as first-line therapy for *BRAF* V600E metastatic colorectal cancer patients. In the safety lead-in stage, 60 patients will receive either encorafenib with cetuximab and FOLFIRI or encorafenib with cetuximab and FOLFOX. In the phase III part, encorafenib and cetuximab or the combination of encorafenib with cetuximab and either FOLFIRI or FOLFOX will be administered in experimental arms. The control group will be treated with a chemotherapy doublet or triplet with or without bevacizumab according to investigators' choice.

Enrollment started in January 2021, and the study is expected to be completed in 2026, hopefully enlightening the question of the best first-line therapy for *BRAF* V600E positive colorectal cancer patients.[166]

In conclusion, novel treatment options for *BRAF* V600E metastatic colorectal cancer patients have improved survival and response rates. We recommend testing of dMMR/MSI in all patients to evaluate the option of checkpoint inhibitor treatment. For other patients, FOLFOX with bevacizumab is the current first-line therapy of choice, followed by encorafenib and cetuximab in second line. The addition of established cytotoxic agents could further improve this therapy strategy and may move it to the first-line setting.

### *KRAS inhibitors – drugging the undruggable?*

While *BRAF* mutations are found in only around 5% of metastatic colorectal cancers, *RAS* mutations are present in more than 40%, and in up to 20% of all cancer types.[167] Therefore, targeting this driver gene could potentially benefit a significantly larger number of patients. Of the three different isoforms of *RAS*, *KRAS* mutations are predominant in colorectal cancer with around 40%, *NRAS* mutations occur in approximately 5%, and *HRAS* plays an insignificant role in colorectal cancer.[168] Oncogenic driver mutations occur most often in codons 12, 13, and 61, leading to a lower rate of guanosine triphosphate (GTP) hydrolysis and thereby constitutively activating the pathway. Besides decreased GTPase activity, a change in the affinity to downstream targets, which differs between the mutations, can also play a role in the activity of the mutation.[169] *KRAS* mutations are associated with worse OS and inferior response toward EGFR antibodies (compare above). Directly targeting the RAS protein, however, is difficult given its high affinity to GTP and its lack of hydrophobic pockets, limiting the binding capacity for small molecules. RAS has therefore been considered an undruggable gene for decades.[170] The most promising attempt was the development of allele-specific inhibitors of the G12 C mutation in the last years. This is based on the relatively strong GTPase activity in G12 C-mutated *KRAS* compared with other *KRAS* mutations.[169] In a fragment-based screen, a pocket was found in which a compound could bind to a reactive cysteine, stabilizing the guanosine diphosphate (GDP) bound (inactive) state of KRAS and thereby leading to decreased KRAS activity.[171] One big advantage of this mode of action is the selectivity toward mutated RAS because only the mutated protein contains a cysteine.[171] Two inhibitors of the G12 C-mutated *KRAS* have been investigated in clinical trials: Sotorasib (AMG510) was the first G12 C-specific KRAS inhibitor tested in a basket trial of 129 patients, 42 of them with colorectal cancer. In the colorectal cancer subgroup, 7.1% had a confirmed response while the disease control rate was 73.8%.[172] Another inhibitor, adagrasib, was tested in a phase I/II trial as monotherapy or in combination with cetuximab. In 45 colorectal cancer patients evaluable for analysis with adagrasib monotherapy, 22% had a confirmed response while the disease control rate was 87% and PFS was 5.6 months. The combination therapy with cetuximab led to 43% response rate and 100% disease control rate in 28 patients[173,174] Thus, both studies showed promising activity in pretreated patients. Further G12 C inhibitors JNJ-74699157, GDC-6036, and JDQ433 are currently being tested in similar trials with colorectal cancer patients (NCT04006301, NCT04449874, and NCT04699188). In some of these trials, combinations with EGFR antibodies or immune checkpoint inhibitors are evaluated since *in vitro* data have shown that G12 C inhibitors are able to increase the effect of targeted therapy and immunotherapy.[175]

Since the G12 C mutation does only occur in about 3% of colorectal cancers, there is still a lack of therapies for more common KRAS mutations.[175,176] A possible solution could be inhibitors that prevent the interaction between the KRAS molecule and SOS, which is mainly responsible for RAS activation. This principle was shown to be effective *in vitro* in fragment-based screens. By binding into a certain pocket in the KRAS protein, the interaction with SOS is prevented, leading to significant decrease in KRAS activity.[177,178] Similar results were obtained for the KRAS inhibitor BI-2852 that could bind to KRAS in nanomolar levels.[179] The compound BAY-293 was found to specifically inhibit the interaction of KRAS and SOS1 at picomolar concentrations, suggesting it as a promising candidate for further investigation.[180] Another SOS1 interaction inhibitor, BI1701963, is currently under investigation in two clinical trials. First, in combination with the MEK inhibitor trametinib in different solid tumors with *KRAS* mutation (NCT04111458), and second, in combination with irinotecan specifically in colorectal cancer

**Table 2.** Therapy targets and molecular predictive markers in metastatic colorectal cancer.

| Target/marker | Frequency (%) | Drug(s) | Type of marker |
|---|---|---|---|
| BRAF V600E | 5 | Encorafenib + cetuximab | Positive predictive |
| MSI/dMMR | 5–10 | Checkpoint inhibitors | Positive predictive |
| NTRK | <1 | Entrectinib, larotrectinib | Positive predictive |
| HER2 amplification | 2.5 | Trastuzumab + pertuzumab, Trastuzumab deruxtecan, Trastuzumab emtansine | Positive predictive |
| KRAS/NRAS | 40 | Cetuximab, panitumumab | Negative predictive |
| Right colon | 20 | Cetuximab, panitumumab | Negative predictive |

patients (NCT04627142). The most important challenge of this approach is the non-specificity of the compounds toward mutant KRAS.[177,179,180] BAY-293, for example, inhibits the proliferation of KRAS WT cells in lower concentrations than in KRAS mutant cells,[180] therefore the toxicity profiles of the compounds in ongoing clinical trials are awaited with great interest.

### Targeting WEE1 in *KRAS*- and *TP53*-mutated tumors

Adavosertib is an inhibitor of WEE1, a tyrosine kinase that is involved in cell-cycle regulation.[181] Based on preclinical considerations of a potentially increased sensitivity of tumors with *TP53* and *KRAS* mutations due to DNA replication aberrations, the drug was tested as maintenance therapy in Arm C of the FOCUS4 platform trial. This setting was likely inspired by trials of other DNA damage response targeting agents, such as PARP-inhibitors, that have, for instance, recently been approved as maintenance therapy after platinum-based induction in *BRCA*-mutated pancreatic cancer.[182] Patients with stable disease or response to first-line induction chemotherapy (approx. 2/3 platinum-based) were randomized to receive adavosertib ($N=44$) or active monitoring ($N=25$). Adavosertib improved the primary end-point PFS (median 3.61 vs 1.87 months), thereby making it an interesting candidate for a larger phase III trial, even though no improvement of OS was observed in this cohort. Other efforts to therapeutically exploit DNA damage response in colorectal cancer have been investigated in preclinical studies and in a few trials testing PAPR inhibitors (olaparib, veliparib) as mono- or in combination therapies[183] No meaningful clinical benefit has been reported in patient cohorts so far. These were, however, mainly unstratified with respect to homologous recombination defects. Further studies are needed to find out if translation of targeting DNA damage response into the clinical setting of colorectal cancer treatment will be successful.

### Targeting rare cancer drivers

Recently, molecular characterization of tumors by whole exome and even whole genome sequencing has greatly increased our knowledge of recurrent mutations and structural aberrations, also in colorectal cancer.[22–24,184,185] Accordingly, several studies are testing the efficacy of specific inhibitors in mainly tumor-type agnostic 'precision medicine' trials and molecular tumor boards.[186–192] However, clinical benefits of this genomics-based therapy stratification remain unproven. To date, only a small fraction of oncogenic driver genes with low prevalence can be targeted by specific inhibitors; hence, only few patients with tumors harboring these alterations can benefit. Table 2 summarizes currently available predictive markers for precision therapy of colorectal cancer. As discussed above, *RAS* mutations and right-sided primary tumor location represent negative predictive factors for EGFR antibody treatment, while MSI-high, *HER2* amplification, and *BRAF* V600E mutations are predictive for response toward specific treatments.

In addition, neurotrophic receptor tyrosine kinase (*NTRK*) gene fusions, very rare targetable alterations, can be found in colorectal cancer. This family of *NTRK1–3* genes is normally involved in physiological regulation of the nervous system; however, fusion of the kinase domain of these genes with a variety of partners (including *RET*,

*MET, ERBB2, FGFR*, and others) in chromosomal rearrangements can lead to potent and ligand-independent cancer drivers (reviewed here).[193] In colorectal cancer, *NTRK* fusions occur in 1–2% of cases and can be detected by next-generation sequencing. Importantly, the efficacy of the available (and FDA/EMA-approved) inhibitors, larotrectinib and entrectinib, is very high according to recent data.[193] Larotrectinib has been evaluated in 55 mainly pretreated patients with diverse tumor types (four of them colon cancers) with a response rate of 75% and a 1-year PFS of 55%.[194] Furthermore, preliminary results of three ongoing trials evaluating entrectinib reported that 57% of 54 patients had an objective response with a median duration of 10 months.[195] Thus, given the putative high efficacy of direct inhibition, patients who are refractory toward first- and second-line therapies should be offered testing for NTRK fusions, especially if no other drivers (*RAS*/*RAF*/*HER2*) have been detected.

## Consensus molecular subtypes: do they have therapeutic implications?

Beyond analysis of mutations or structural genomic aberrations as predictive markers or therapy targets, transcriptome-based analyses aiming to improve therapeutic patient stratification by establishing new molecular classification systems of colorectal cancer have been published in recent years.[196–201] For better clinical standardization and translation, the classification systems were unified into the 'consensus molecular subtypes' (CMS) by an international consortium.[202] CMS categorizes colorectal cancers into four subtypes with distinct tumor biology: CMS1 (MSI/immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal).[202] The subtypes were related to previously known genomic, epigenetic, histological, and clinical features of colorectal cancer.[145,202] To this end, CMS1 largely overlapped with dMMR/MSI cancers, a high degree of immune cell infiltration, low tumor differentiation, a higher frequency of BRAF mutations, hypermethylation, and location of the primary tumor in the proximal colon. In contrast, CMS2 and CMS4 had many copy number variations, were pMMR, and low in methylation rate. CMS2, representing the 'canonical subtype', was also found to be associated with WNT and MYC activation, and left-sided location, while CMS4 was related to mesenchymal signatures, TGF-beta activation, and extracellular matrix remodeling, histologically characterized by a strong desmoplastic reaction.[145,202] Finally, CMS3 was characterized by marked metabolic reprogramming, including activation of lipogenesis, while MSI status was mixed and copy number variations and methylation status were low.[145,202,203] CMS1 is associated with the worst prognosis in Stage IV disease, while CMS2–4 have better overall outcome in this stage.[204,205]

CMS has also been studied as a predictive signature for therapy selection in metastatic cancers. Here, CMS1 is most likely associated with sensitivity to checkpoint inhibitor treatment due to its association with MSI/hypermutation. Published data are partially conflicting with respect to prediction of survival on EGFR and VEGF antibody combination therapies. Retrospective transcriptomic analysis for CMS classification of tumors from the phase III FIRE-3 and CALGB/SWOG 80405 clinical trials have been performed.[204,205] These studies had compared the addition of bevacizumab or cetuximab to doublet chemotherapy as first-line treatment. In the CALGB/SWOG 80405 analysis, CMS1 patients treated with bevacizumab had better OS than those treated with cetuximab, while the CMS2 patients benefited more from cetuximab therapy. According to the FIRE-3 study data, OS was comparable in CMS1 and CMS2 subgroups, independent of targeted therapy, while CSM3 and CSM4 both favored cetuximab with a longer OS. In addition, retrospective analysis of AGITG MAX trial data suggested CMS2 and possibly CMS3 tumors to benefit from bevacizumab in addition to capecitabine chemotherapy, compared with other CMS.[206] Other retrospective data suggested a worse outcome associated with anti-EGFR antibodies in CMS1 and a favorable outcome in CMS2.[207] Differences of CMS predictive values found in these studies have been attributed to different chemotherapy backbones used in the trial populations, interaction of chemotherapy with targeted therapies, the tumor microenvironment, and differences in therapy sequence.[208] Interestingly, in a different approach classifying molecular subtypes based on gene copy number variations instead of gene expression, Smeets et al.[209] found that tumors with a high or intermediate degree of chromosomal instability had improved outcome after bevacizumab combination therapy, while the subgroup with low degree of copy number variations (corresponding CMS1 or dMMR/MSI/hypermutated tumors) did not benefit from bevacizumab treatment. With

respect to conventional chemotherapies, improved outcome of irinotecan- versus oxaliplatin-based combination treatment has been reported for CMS4 tumors.[207] Notably, studies in preclinical models of colorectal cancer suggest associations of CMS with response to other specific anticancer drugs, including oxaliplatin,[210] HSP-90 inhibitors,[211] birinapant,[212] or YM-155,[213] indicating that the CMS classification may have predictive potential for specific substances.

Thus, cetuximab might be beneficial in CMS2–4 and irinotecan specifically in CMS4 tumors, while the situation for bevacizumab seems less clear. Due to this rather preliminary data and relatively laborious methodology, CMS currently has no application in routine clinical practice. Further data, favorably from prospective trials, are needed to define the future predictive value of CMS in metastatic colorectal cancer.

## Functional precision medicine – science fiction or realistic path to explore?

Due to the low prevalence of druggable mutations and molecular biomarkers for drug efficacy, testing of drugs *ex vivo* in suitable model systems for personalized predictions has been proposed to complement molecular genetic testing and referred to as 'functional precision medicine'.[214] Tumor models, such as patient-derived xenografts (PDX) or patient-derived organoids (PDO), are used for preclinical drug screenings, co-clinical trials, and also personalized therapy predictions.[215,216] PDX are mouse models, in which tumor cells or fragments obtained from tumors of cancer patients are implanted orthotopically or heterotopically (for instance, subcutaneously or into the renal capsule) into immunodeficient mice.[217] These implants form tumors within several weeks and have been shown to resemble their origin, so that PDX can be used for drug testing in a personalized 'mouse avatar'.[215,218] Some studies have demonstrated high correlations of treatment response between patient and PDX;[215,216,219] however, the technology is laborious, engraftment rates differ between patients and tumors, engraftment time can be too long for personalized testing, and ethics are controversial.

Organoids are stem cell-derived three-dimensional cell cultures that grow in extracellular matrix with the help of culture medium supplemented with stem cell niche factors.[220–222] Similar to PDX, PDOs resemble their origin, that is, tumors or healthy epithelial tissues, with respect to molecular and morphological features.[223–225] They can be established from colorectal cancers with high efficiency (approx. 70%),[226–228] kept in culture long term and can be expanded for biobanking or drug profiling studies.[225,229,230] In addition, recent studies have reported a high degree of correlation between patients' and matched organoid drug response, when treated with the same substances.[226–228] However, the only completed interventional trial (SENSOR) testing organoid-predicted precision treatments in 'last-line' colorectal cancer patients failed to show a meaningful clinical benefit.[231] Further development of the model, and standardization and benchmarking with clinical response might be necessary for PDOs to harvest clinical benefit. Nevertheless, both PDX and PDOs are invaluable tools depicting the diversity of colorectal cancer in the laboratory for preclinical development of novel treatments and for biomarker research.

## Conclusion and outlook

In conclusion, the therapy of metastatic colorectal cancer has greatly improved in recent years. Some patients can be cured by resection of metastases, especially in combination with advanced chemotherapy and targeted therapy protocols. These treatments need to be carefully tailored to molecular (RAS/RAF/MSI) and clinical (sidedness, performance status) predictive markers. Beyond, specific subgroups, such as patients with dMMR/MSI tumors, tumors with *HER2* amplification, *BRAF* V600E mutation, and *NTRK* fusion, benefit from immunotherapy or targeted therapies, respectively. Therefore, analyzing these molecular characteristics of tumors is necessary to allow optimal patient care. More detailed molecular characterization, eventually in combination with functional testing using advanced preclinical models, may indicate further treatment options for advanced colorectal cancer patients in the future. However, most oncogenic drivers of colorectal cancer are currently not druggable, so that precision treatments for the majority of patients are not foreseeable and would require breakthroughs in basic research. Further developments of immunotherapy appear inciting and promising areas in oncological research in general. In this regard, exploiting the immune system in yet immunologically cold tumors seems

to be a holy grail of preclinical and translational research in coming years.

## Authors' note

Julian E. Riedesser and Matthias P. Ebert now affiliated to DKFZ-Hector Cancer Institute at University Medical Center Mannheim, Mannheim, Germany.

## Author contributions

**Julian E. Riedesse:** Conceptualization; Investigation; Writing – original draft; Writing – review & editing.

**Matthias P. Ebert:** Conceptualization; Investigation; Supervision; Writing – review & editing.

**Johannes Betge:** Conceptualization; Investigation; Supervision; Writing – original draft; Writing – review & editing.

## Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: J.B. is supported by the Hector Foundation II, Weinheim, Germany.

## ORCID iD

Johannes Betge https://orcid.org/0000-0001-9549-1866

## References

1. Sung H, Ferlay J, Siegel RL, *et al.* 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.
2. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. *Ca Cancer J Clin* 2019; 69: 7–34.
3. de Gramont A, Figer A, Seymour M, *et al.* Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. *J Clin Oncol* 2000; 18: 2938–2947.
4. Goldberg RM, Sargent DJ, Morton RF, *et al.* A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. *J Clin Oncol* 2003; 22: 23–30.
5. Gustavsson B, Carlsson G, Machover D, *et al.* A review of the evolution of systemic chemotherapy in the management of colorectal cancer. *Clin Colorectal Cancer* 2015; 14: 1–10.
6. Douillard J, Cunningham D, Roth A, *et al.* Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. *Lancet* 2000; 355: 1041–1047.
7. Saltz LB, Cox JV, Blanke C, *et al.* Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2000; 343: 905–914.
8. Hurwitz HI, Fehrenbacher L, Hainsworth JD, *et al.* Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. *J Clin Oncol* 2005; 23: 3502–3508.
9. Kabbinavar FF, Schulz J, McCleod M, *et al.* Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. *J Clin Oncol* 2005; 23: 3697–3705.
10. Saltz LB, Clarke S, Díaz-Rubio E, *et al.* Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008; 26: 2013–2019.
11. 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.
12. Cutsem EV, 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: 3499–3506.
13. Cutsem EV, Köhne C-H, Hitre E, *et al.* Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N Engl J Med* 2009; 360: 1408–1417.
14. Douillard J-Y, 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: 4697–4705.

15. Cutsem EV, Köhne C-H, Láng I, *et al*. Cetuximab plus irinotecan, fluorouracil, and leucovorin as first-line treatment for metastatic colorectal cancer: updated analysis of overall survival according to tumor KRAS and BRAF mutation status. *J Clin Oncol* 2011; 29: 2011–2019.
16. Mayer RJ, Cutsem EV, Falcone A, *et al*. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med* 2015; 372: 1909–1919.
17. Grothey A, Cutsem EV, 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: 303–312.
18. Jones R. Cytotoxic chemotherapy: clinical aspects. *Medicine* 2016; 44: 25–29.
19. Nordlinger B, Van Cutsem E, Gruenberger T, *et al*. Combination of surgery and chemotherapy and the role of targeted agents in the treatment of patients with colorectal liver metastases: recommendations from an expert panel. *Ann Oncol* 2009; 20: 985–992.
20. Cutsem EV, Cervantes A, Adam R, *et al*. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 2016; 27: 1386–1422.
21. Wood LD, Parsons DW, Jones S, *et al*. The genomic landscapes of human breast and colorectal cancers. *Science* 2007; 318: 1108–1113.
22. Muzny DM, Bainbridge MN, Chang K, *et al*. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; 487: 330–337.
23. Kandoth C, McLellan MD, Vandin F, *et al*. Mutational landscape and significance across 12 major cancer types. *Nature* 2013; 502: 333–339.
24. Campbell PJ, Getz G, Korbel JO, *et al*. Pan-cancer analysis of whole genomes. *Nature* 2020; 578: 82–93.
25. Imanishi M, Yamamoto Y, Hamano Y, *et al*. Efficacy of adjuvant chemotherapy after resection of pulmonary metastasis from colorectal cancer: a propensity score–matched analysis. *Eur J Cancer* 2019; 106: 69–77.
26. Nordlinger B, Sorbye H, Glimelius B, *et al*. Perioperative FOLFOX4 chemotherapy and surgery versus surgery alone for resectable liver metastases from colorectal cancer (EORTC 40983): long-term results of a randomised, controlled, phase 3 trial. *Lancet Oncol* 2013; 14: 1208–1215.
27. Fong Y, Fortner J, Sun RL, *et al*. Clinical score for predicting recurrence after hepatic resection for metastatic colorectal cancer. *Ann Surg* 1999; 230: 309–318; discussion 318.
28. Folprecht G, Gruenberger T, Bechstein WO, *et al*. Tumour response and secondary resectability of colorectal liver metastases following neoadjuvant chemotherapy with cetuximab: the CELIM randomised phase 2 trial. *Lancet Oncol* 2010; 11: 38–47.
29. Adam R, Delvart V, Pascal G, *et al*. Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy. *Ann Surg* 2004; 240: 644–657; discussion 657.
30. Folprecht G, Gruenberger T, Bechstein W, *et al*. Survival of patients with initially unresectable colorectal liver metastases treated with FOLFOX/cetuximab or FOLFIRI/cetuximab in a multidisciplinary concept (CELIM study). *Ann Oncol* 2014; 25: 1018–1025.
31. Ye LC, Liu TS, Ren L, *et al*. Randomized controlled trial of cetuximab plus chemotherapy for patients with KRAS wild-type unresectable colorectal liver-limited metastases. *J Clin Oncol* 2013; 31: 1931–1938.
32. Gruenberger T, Bridgewater J, Chau I, *et al*. Bevacizumab plus mFOLFOX-6 or FOLFOXIRI in patients with initially unresectable liver metastases from colorectal cancer: the OLIVIA multinational randomised phase II trial. *Ann Oncol* 2015; 26: 702–708.
33. Goldberg RM, Rothenberg ML, Van Cutsem E, *et al*. The continuum of care: a paradigm for the management of metastatic colorectal cancer. *Oncologist* 2007; 12: 38–50.
34. Tournigand C, André T, Achille E, *et al*. FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. *J Clin Oncol* 2003; 22: 229–237.
35. Colucci G, Gebbia V, Paoletti G, *et al*. Phase III randomized trial of FOLFIRI versus FOLFOX4 in the treatment of advanced colorectal cancer: a multicenter study of the Gruppo Oncologico Dell'Italia Meridionale. *J Clin Oncol* 2005; 23: 4866–4875.
36. de Gramont A, Bosset JF, Milan C, *et al*. Randomized trial comparing monthly low-dose leucovorin and fluorouracil bolus with bimonthly high-dose leucovorin and fluorouracil bolus plus continuous infusion for advanced colorectal cancer: a French intergroup study. *J Clin Oncol* 1997; 15: 808–815.

37. Cassidy J, Clarke S, Díaz-Rubio E, *et al.* Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. *J Clin Oncol* 2008; 26: 2006–2012.

38. Fuchs CS, Marshall J, Mitchell E, *et al.* 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.

39. Maughan TS, Adams RA, Smith CG, *et al.* Addition of cetuximab to oxaliplatin-based first-line combination chemotherapy for treatment of advanced colorectal cancer: results of the randomised phase 3 MRC COIN trial. *Lancet* 2011; 377: 2103–2114.

40. Deenen MJ, Meulendijks D, Cats A, *et al.* Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. *J Clin Oncol* 2015; 34: 227–234.

41. Henricks LM, Lunenburg CATC, de Man FM, *et al.* DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* 2018; 19: 1459–1467.

42. Schmiegel W, Buchberger B, Follmann M, *et al.* S3-Leitlinie Kolorektales Karzinom. *Z Gastroenterol* 2017; 55: 1344–1498.

43. Benson AB, Venook AP, Al-Hawary MM, *et al.* Colon cancer, version 2.2021, NCCN clinical practice guidelines in oncology. *J National Compr Cancer Netw Jnccn* 2021; 19: 329–359.

44. Falcone A, Ricci S, Brunetti I, *et al.* 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.

45. Loupakis F, Cremolini C, Masi G, *et al.* Initial therapy with FOLFOXIRI and bevacizumab for metastatic colorectal cancer. *N Engl J Med* 2014; 371: 1609–1618.

46. Souglakos J, Androulakis N, Syrigos K, *et al.* FOLFOXIRI (folinic acid, 5-fluorouracil, oxaliplatin and irinotecan) vs FOLFIRI (folinic acid, 5-fluorouracil and irinotecan) as first-line treatment in metastatic colorectal cancer (MCC): a multicentre randomised phase III trial from the Hellenic Oncology Research Group (HORG). *Br J Cancer* 2006; 94: 798–805.

47. 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: 1306–1315.

48. Heinemann V, von Weikersthal LF, Decker T, *et al.* 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.

49. Schwartzberg LS, Rivera F, Karthaus M, *et al.* 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.

50. Heinemann V, Rivera F, O'Neil BH, *et al.* A study-level meta-analysis of efficacy data from head-to-head first-line trials of epidermal growth factor receptor inhibitors versus bevacizumab in patients with RAS wild-type metastatic colorectal cancer. *Eur J Cancer* 2016; 67: 11–20.

51. Stintzing S, Tejpar S, Gibbs P, *et al.* Understanding the role of primary tumour localisation in colorectal cancer treatment and outcomes. *Eur J Cancer* 2017; 84: 69–80.

52. Tejpar S, Stintzing S, Ciardiello F, *et al.* Prognostic and predictive relevance of primary tumor location in patients with RAS wild-type metastatic colorectal cancer: retrospective analyses of the CRYSTAL and FIRE-3 trials. *JAMA Oncol*. Epub ahead of print 1 January 2016. DOI: 10.1001/jamaoncol.2016.3797.

53. Arnold D, Lueza B, Douillard J-Y, *et al.* Prognostic and predictive value of primary tumour side in patients with RAS wild-type metastatic colorectal cancer treated with chemotherapy and EGFR directed antibodies in six randomized trials. *Ann Oncol* 2017; 28: 1713–1729.

54. Hegewisch-Becker S, Graeven U, Lerchenmüller CA, *et al.* Maintenance strategies after first-line oxaliplatin plus fluoropyrimidine plus bevacizumab for patients with metastatic colorectal cancer (AIO 0207): a randomised, non-inferiority, open-label, phase 3 trial. *Lancet Oncol* 2015; 16: 1355–1369.

55. Simkens LHJ, Tinteren H, van May A, *et al.* Maintenance treatment with capecitabine and bevacizumab in metastatic colorectal cancer (CAIRO3): a phase 3 randomised controlled trial of the Dutch Colorectal Cancer Group. *Lancet* 2015; 385: 1843–1852.

56. Zhao L, Wang J, Li H, *et al*. Meta-analysis comparing maintenance strategies with continuous therapy and complete chemotherapy-free interval strategies in the treatment of metastatic colorectal cancer. *Oncotarget* 2015; 7: 33418–33428.

57. Esin E and Yalcin S. Maintenance strategy in metastatic colorectal cancer: a systematic review. *Cancer Treat Rev* 2016; 42: 82–90.

58. Berry SR, Cosby R, Asmis T, *et al*. Continuous versus intermittent chemotherapy strategies in metastatic colorectal cancer: a systematic review and meta-analysis. *Ann Oncol* 2015; 26: 477–485.

59. Modest DP, Pant S and Sartore-Bianchi A. Treatment sequencing in metastatic colorectal cancer. *Eur J Cancer* 2019; 109: 70–83.

60. Giordano G, Parcesepe P, Bruno G, *et al*. Evidence-based second-line treatment in RAS wild-type/mutated metastatic colorectal cancer in the precision medicine era. *Int J Mol Sci* 2021; 22: 7717.

61. Bennouna J, Sastre J, Arnold D, *et al*. Continuation of bevacizumab after first progression in metastatic colorectal cancer (ML18147): a randomised phase 3 trial. *Lancet Oncol* 2013; 14: 29–37.

62. 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: 724–730.

63. 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: 1539–1544.

64. Sobrero AF, 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: 2311–2319.

65. 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: 4706–4713.

66. Zaniboni A and Formica V. The best. first. anti-EGFR before anti-VEGF, in the first-line treatment of RAS wild-type metastatic colorectal cancer: from bench to bedside. *Cancer Chemother Pharmacol* 2016; 78: 233–244.

67. Modest DP, Stintzing S, Weikersthal LF, *et al*. Impact of subsequent therapies on outcome of the FIRE-3/AIO KRK0306 trial: first-line therapy with FOLFIRI plus cetuximab or bevacizumab in patients with KRAS wild-type tumors in metastatic colorectal cancer. *J Clin Oncol* 2015; 33: 3718–3726.

68. Moretto R, Cremolini C, Rossini D, *et al*. Location of primary tumor and benefit from anti-epidermal growth factor receptor monoclonal antibodies in patients with RAS and BRAF wild-type metastatic colorectal cancer. *Oncologist* 2016; 21: 988–994.

69. Brulé SY, Jonker DJ, Karapetis CS, *et al*. Location of colon cancer (right-sided versus left-sided) as a prognostic factor and a predictor of benefit from cetuximab in NCIC CO.17. *Eur J Cancer* 2015; 51: 1405–1414.

70. Ushida Y, Shinozaki E, Chin K, *et al*. Clinical outcomes of anti-EGFR antibody treatment for right-sided colon cancer patients without RAS, BRAF, and PIK3CA mutations in the later line. *J Clin Oncol* 2018; 36: 812–812.

71. Kuboki Y, Nishina T, Shinozaki E, *et al*. 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.

72. Pfeiffer P, Yilmaz M, Möller S, *et al*. TAS-102 with or without bevacizumab in patients with chemorefractory metastatic colorectal cancer: an investigator-initiated, open-label, randomised, phase 2 trial. *Lancet Oncol* 2020; 21: 412–420.

73. Vogel A, Hofheinz RD, Kubicka S, *et al*. Treatment decisions in metastatic colorectal cancer – beyond first and second line combination therapies. *Cancer Treat Rev* 2017; 59: 54–60.

74. Ribas A and Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science* 2018; 359: 1350–1355.

75. Yi M, Jiao D, Xu H, *et al*. Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors. *Mol Cancer* 2018; 17: 129.

76. Rosenbaum MW, Bledsoe JR, Morales-Oyarvide V, *et al*. PD-L1 expression in colorectal cancer is associated with microsatellite instability, BRAF mutation, medullary morphology and cytotoxic tumor-infiltrating lymphocytes. *Mod Pathol* 2016; 29: 1104–1112.

77. Fearon ER. Molecular genetics of colorectal cancer. *Ann Rev Pathol Mech Dis* 2011; 6: 479–507.

78. Le DT, Uram JN, Wang H, *et al*. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 2015; 372: 2509–2520.

79. Le DT, Durham JN, Smith KN, *et al.* Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* 2017; 357: 409–413.

80. Hirano H, Takashima A, Hamaguchi T, *et al.* Current status and perspectives of immune checkpoint inhibitors for colorectal cancer. *Jpn J Clin Oncol* 2020; 51: 10–19.

81. Jung G, Benítez-Ribas D, Sánchez A, *et al.* Current treatments of metastatic colorectal cancer with immune checkpoint inhibitors—2020 update. *J Clin Med* 2020; 9: 3520.

82. Le DT, Kim TW, Cutsem EV, *et al.* Phase II open-label study of pembrolizumab in treatment-refractory, microsatellite instability–high/mismatch repair–deficient metastatic colorectal cancer: KEYNOTE-164. *J Clin Oncol* 2020; 38: 11–19.

83. 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: 1182–1191.

84. 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: 773–779.

85. Kim JH, Kim SY, Baek JY, *et al.* A phase II study of avelumab monotherapy in patients with mismatch repair-deficient/microsatellite instability-high or POLE-mutated metastatic or unresectable colorectal cancer. *Cancer Res Treat* 2020; 52: 1135–1144.

86. Segal NH, Wainberg ZA, Overman MJ, *et al.* Safety and clinical activity of durvalumab monotherapy in patients with microsatellite instability–high (MSI-H) tumors. *J Clin Oncol* 2019; 37: 670–670.

87. 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: 673–673.

88. Lenz H-J, Cutsem EV, Limon ML, *et al.* First-line nivolumab plus low-dose ipilimumab for microsatellite instability-high/mismatch repair-deficient metastatic colorectal cancer: the phase II checkmate 142 study. *J Clin Oncol.* Epub ahead of print 12 October 2021. DOI: 10.1200/JCO.21.01015.

89. André T, Shiu K-K, Kim TW, *et al.* Pembrolizumab in microsatellite-instability–high advanced colorectal cancer. *N Engl J Med* 2020; 383: 2207–2218.

90. Andre T, Shiu K-K, Kim TW, *et al.* Final overall survival for the phase III KN177 study: pembrolizumab versus chemotherapy in microsatellite instability-high/mismatch repair deficient (MSI-H/dMMR) metastatic colorectal cancer (mCRC). *J Clin Oncol* 2021; 39: 3500–3500.

91. Pecci F, Cantini L, Bittoni A, *et al.* Beyond microsatellite instability: evolving strategies integrating immunotherapy for microsatellite stable colorectal cancer. *Curr Treat Option on* 2021; 22: 69.

92. Motz GT, Santoro SP, Wang L-P, *et al.* Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat Med* 2014; 20: 607–615.

93. Voron T, Colussi O, Marcheteau E, *et al.* VEGF-A modulates expression of inhibitory checkpoints on CD8+ T cells in tumors. *J Exp Med* 2015; 212: 139–148.

94. Wallin JJ, Bendell JC, Funke R, *et al.* Atezolizumab in combination with bevacizumab enhances antigen-specific T-cell migration in metastatic renal cell carcinoma. *Nat Commun* 2016; 7: 12624.

95. Roland CL, Dineen SP, Lynn KD, *et al.* Inhibition of vascular endothelial growth factor reduces angiogenesis and modulates immune cell infiltration of orthotopic breast cancer xenografts. *Mol Cancer Ther* 2009; 8: 1761–1771.

96. Finn RS, Qin S, Ikeda M, *et al.* Atezolizumab plus bevacizumab in unresectable hepatocellular carcinoma. *N Engl J Med* 2020; 382: 1894–1905.

97. Reck M, Shankar G, Lee A, *et al.* Atezolizumab in combination with bevacizumab, paclitaxel and carboplatin for the first-line treatment of patients with metastatic non-squamous non-small cell lung cancer, including patients with EGFR mutations. *Expert Rev Resp Med* 2019; 14: 1–12.

98. Rini BI, Powles T, Atkins MB, *et al.* Atezolizumab plus bevacizumab versus sunitinib in patients with previously untreated metastatic renal cell carcinoma (IMmotion151): a multicentre, open-label, phase 3, randomised controlled trial. *Lancet* 2019; 393: 2404–2415.

99. Mettu NB, Twohy E, Ou F-S, *et al.* BACCI: a phase II randomized, double-blind, multicenter, placebo-controlled study of capecitabine (C) bevacizumab (B) plus atezolizumab (A) or placebo (P) in refractory metastatic colorectal cancer (mCRC): an ACCRU network study. *Ann Oncol* 2019; 30: v203.

100. Fukuoka S, Hara H, Takahashi N, *et al*. 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.

101. Cousin S, Cantarel C, Guegan J-P, *et al*. Regorafenib-avelumab combination in patients with microsatellite stable colorectal cancer (REGOMUNE): a single-arm, open-label, phase II trial. *Clin Cancer Res* 2021; 27: 2139–2147.

102. Damato A, Berselli A, Iachetta F, *et al*. Preliminary safety analysis of phase II open-label NIVACOR trial (GOIRC-03-2018) in patients with advanced colorectal cancer RAS or BRAF mutated. *J Clin Oncol* 2021; 39: 37–37.

103. Eng C, Kim TW, Bendell J, *et al*. Atezolizumab with or without cobimetinib versus regorafenib in previously treated metastatic colorectal cancer (IMblaze370): a multicentre, open-label, phase 3, randomised, controlled trial. *Lancet Oncol* 2019; 20: 849–861.

104. Nigro CL, Ricci V, Vivenza D, *et al*. Evaluation of antibody-dependent cell-mediated cytotoxicity activity and cetuximab response in KRAS wild-type metastatic colorectal cancer patients. *World J Gastrointest Oncol* 2016; 8: 222.

105. Stein A, Binder M, Goekkurt E, *et al*. Avelumab and cetuximab in combination with FOLFOX in patients with previously untreated metastatic colorectal cancer (MCRC): final results of the phase II AVETUX trial (AIO-KRK-0216). *J Clin Oncol* 2020; 38: 96–96.

106. Palmer AC and Sorger PK. Combination cancer therapy can confer benefit via patient-to-patient variability without drug additivity or synergy. *Cell* 2017; 171: 1678–1691.e13.

107. Lowenstein EJ, Daly RJ, Batzer AG, *et al*. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* 1992; 70: 431–442.

108. Okabayashi Y, Kido Y, Okutani T, *et al*. Tyrosines 1148 and 1173 of activated human epidermal growth factor receptors are binding sites of Shc in intact cells. *J Biol Chem* 1994; 269: 18674–18678

109. Rozakis-Adcock M, Fernley R, Wade J, *et al*. The SH2 and SH3 domains of mammalian Grb2 couple the EGF receptor to the Ras activator mSos1. *Nature* 1993; 363: 83–85.

110. Bonfini L, Karlovich C, Dasgupta C, *et al*. The son of sevenless gene product: a putative activator of Ras. *Science* 1992; 255: 603–606.

111. Buday L and Downward J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* 1993; 73: 611–620.

112. Boriack-Sjodin PA, Margarit SM, Bar-Sagi D, *et al*. The structural basis of the activation of Ras by Sos. *Nature* 1998; 394: 337–343.

113. Simanshu DK, Nissley DV and McCormick F. RAS proteins and their regulators in human disease. *Cell* 2017; 170: 17–33.

114. Wellbrock C, Karasarides M and Marais R. The RAF proteins take centre stage. *Nat Rev Mol Cell Biol* 2004; 5: 875–885.

115. Vojtek AB, Hollenberg SM and Cooper JA. Mammalian Ras interacts directly with the serine/threonine kinase raf. *Cell* 1993; 74: 205–214.

116. Roskoski R. MEK1/2 dual-specificity protein kinases: structure and regulation. *Biochem Biophys Res Commun* 2012; 417: 5–10.

117. Yoon S and Seger R. The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. *Growth Factors* 2009; 24: 21–44.

118. Arteaga CL and Engelman JA. ERBB receptors: from oncogene discovery to basic science to mechanism-based cancer therapeutics. *Cancer Cell* 2014; 25: 282–303.

119. Oh D-Y and Bang Y-J. HER2-targeted therapies – a role beyond breast cancer. *Nat Rev Clin Oncol* 2020; 17: 33–48.

120. Meric-Bernstam F, Johnson A, Dumbrava EEI, *et al*. Advances in HER2-targeted therapy: novel agents and opportunities beyond breast and gastric cancer. *Clin Cancer Res* 2019; 25: 2033–2041.

121. Pegram MD, Konecny G, Slamon DJ, *et al*. The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (Trastuzumab) therapy for breast cancer. *Cancer Treat Res* 2000; 103: 57–75.

122. Hendriks BS, Opresko LK, Wiley HS, *et al*. Quantitative analysis of HER2-mediated effects on HER2 and epidermal growth factor receptor endocytosis distribution of homo-AND heterodimers depends on relative HER2 levels. *J Biol Chem* 2003; 278: 23343–23351.

123. Zehir A, Benayed R, Shah RH, *et al*. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients. *Nat Med* 2017; 23: 703–713.

124. Pahuja KB, Nguyen TT, Jaiswal BS, *et al*. Actionable activating oncogenic ERBB2/HER2 transmembrane and juxtamembrane domain mutations. *Cancer Cell* 2018; 34: 792–806.e5.

125. Wolff AC, Hammond MEH, Allison KH, *et al*. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *Arch Pathol Lab Med* 2018; 142: 1364–1382.

126. Slamon DJ, Leyland-Jones B, Shak S, *et al*. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001; 344: 783–792.

127. Bang Y-J, Cutsem EV, Feyereislova A, *et al*. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; 376: 687–697.

128. 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: 1358–1373.

129. Greally M, Kelly CM and Cercek A. HER2: an emerging target in colorectal cancer. *Curr Probl Cancer* 2018; 42: 560–571.

130. Sartore-Bianchi A, Amatu A, Porcu L, *et al*. HER2 positivity predicts unresponsiveness to EGFR-targeted treatment in metastatic colorectal cancer. *Oncologist* 2019; 24: 1395–1402.

131. Bertotti A, Migliardi G, Galimi F, *et al*. A molecularly annotated platform of patient-derived xenografts ('xenopatients') identifies HER2 as an effective therapeutic target in cetuximab-resistant colorectal cancer. *Cancer Discov* 2011; 1: 508–523.

132. 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: 738–746.

133. Hainsworth JD, 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: 536–542.

134. Phillips GDL, Li G, Dugger DL, *et al*. Targeting HER2-positive breast cancer with trastuzumab-DM1, an antibody-cytotoxic drug conjugate. *Cancer Res* 2008; 68: 9280–9290.

135. Sartore-Bianchi A, Lonardi S, Martino C, *et al*. Pertuzumab and trastuzumab emtansine in patients with HER2-amplified metastatic colorectal cancer: the phase II HERACLES-B trial. *Esmo Open* 2020; 5: e000911.

136. Ogitani Y, Aida T, Hagihara K, *et al*. DS-8201a, a novel HER2-targeting ADC with a novel DNA Topoisomerase I inhibitor, demonstrates a promising antitumor efficacy with differentiation from T-DM1. *Clin Cancer Res* 2016; 22: 5097–5108.

137. Tsurutani J, Iwata H, Krop I, *et al*. Targeting HER2 with trastuzumab deruxtecan: a dose-expansion, phase I study in multiple advanced solid tumors. *Cancer Discov* 2020; 10: 688–701.

138. Li BT, Makker V, Buonocore DJ, *et al*. A multi-histology basket trial of ado-trastuzumab emtansine in patients with HER2 amplified cancers. *J Clin Oncol* 36: 2502–2502.

139. Siena S, Di Bartolomeo M, Raghav K, *et al*. 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.

140. Davies H, Bignell GR, Cox C, *et al*. Mutations of the BRAF gene in human cancer. *Nature* 2002; 417: 949–954.

141. Wan PTC, Garnett MJ, Roe SM, *et al*. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. *Cell* 2004; 116: 855–867.

142. Modest DP, Ricard I, Heinemann V, *et al*. Outcome according to KRAS-, NRAS- and BRAF-mutation as well as KRAS mutation variants: pooled analysis of five randomized trials in metastatic colorectal cancer by the AIO colorectal cancer study group. *Ann Oncol* 2016; 27: 1746–1753.

143. Yaeger R, Cercek A, Chou JF, *et al*. BRAF mutation predicts for poor outcomes after metastasectomy in patients with metastatic colorectal cancer. *Cancer* 2014; 120: 2316–2324.

144. 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: 4623–4632.

145. Dienstmann R, Vermeulen L, Guinney J, *et al*. Consensus molecular subtypes and the evolution

of precision medicine in colorectal cancer. *Nat Rev Cancer* 2017; 17: 79–92.

146. Jones JC, Renfro LA, Al-Shamsi HO, *et al.* Non-V600 BRAF mutations define a clinically distinct molecular subtype of metastatic colorectal cancer. *J Clin Oncol* 2017; 35: 2624–2630.

147. Johnson B, Jin Z, Truty MJ, *et al.* Impact of metastasectomy in the multimodality approach for BRAF V600E metastatic colorectal cancer: the mayo clinic experience. *Oncol* 2018; 23: 128–134.

148. Prasanna T, Wong R, Price T, *et al.* Metastasectomy and BRAF mutation; an analysis of survival outcome in metastatic colorectal cancer. *Curr Prob Cancer* 2020; 45: 100637.

149. Cremolini C, Antoniotti C, Rossini D, *et al.* 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.

150. Cremolini C, Antoniotti C, Stein A, *et al.* 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; 38: 3314–3324.

151. Hyman DM, Puzanov I, Subbiah V, *et al.* Vemurafenib in multiple nonmelanoma cancers with BRAF V600 mutations. *N Engl J Med* 2015; 373: 726–736.

152. Subbiah V, Gervais R, Riely G, *et al.* Efficacy of vemurafenib in patients with non–small-cell lung cancer with BRAF V600 mutation: an open-label, single-arm cohort of the histology-independent VE-BASKET study. *JCO Precis Oncol* 2019; 3: 1–9.

153. Planchard D, Kim TM, Mazieres J, *et al.* Dabrafenib in patients with BRAF V600E-positive advanced non-small-cell lung cancer: a single-arm, multicentre, open-label, phase 2 trial. *Lancet Oncol* 2016; 17: 642–650.

154. Robert C, Grob JJ, Stroyakovskiy D, *et al.* Five-year outcomes with dabrafenib plus trametinib in metastatic melanoma. *N Engl J Med* 2019; 381: 626–636.

155. Chapman PB, Hauschild A, Robert C, *et al.* Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med* 2011; 364: 2507–2516.

156. Hauschild A, Grob J-J, Demidov LV, *et al.* Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. *Lancet* 2012; 380: 358–365.

157. Gomez-Roca CA, Delord J, Robert C, *et al.* Encorafenib (Lgx818), an oral BRAF inhibitor, in patients (Pts) with BRAF V600E metastatic colorectal cancer (Mcrc): results of dose expansion in an open-label, phase 1 study. *Ann Oncol* 2014; 25: iv182.

158. 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: 4032–4038.

159. Corcoran RB, Ebi H, Turke AB, *et al.* EGFR-mediated reactivation of MAPK signaling contributes to insensitivity of BRAF-mutant colorectal cancers to RAF inhibition with vemurafenib. *Cancer Discov* 2012; 2: 227–235.

160. Prahallad A, Sun C, Huang S, *et al.* Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR. *Nature* 2012; 483: 100–103.

161. Hong DS, Morris VK, El Osta B, *et al.* 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.

162. van Geel RMJM, Tabernero J, Elez E, *et al.* A phase Ib dose-escalation study of encorafenib and cetuximab with or without alpelisib in metastatic BRAF-mutant colorectal cancer. *Cancer Discov* 2017; 7: 610–619.

163. 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: 428–443.

164. Kopetz S, Grothey A, Yaeger R, *et al.* Encorafenib, binimetinib, and cetuximab in BRAF V600E–mutated colorectal cancer. *N Engl J Med* 2019; 381: 1632–1643.

165. Grothey A, Tabernero J, Taieb J, *et al.* 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: S242–S243.

166. Kopetz S, Grothey A, Yaeger R, *et al.* BREAKWATER: randomized phase 3 study of encorafenib (enco) + cetuximab (cetux) ± chemotherapy for first-line (1L) treatment (tx) of BRAF V600E-mutant (BRAF

V600E) metastatic colorectal cancer (mCRC). *J Clin Oncol* 2021; 39: TPS3619.

167. Prior IA, Hood FE and Hartley JL. The frequency of ras mutations in cancer. *Cancer Res* 2020; 80: 2969–2974.

168. Serebriiskii IG, Connelly C, Frampton G, *et al.* Comprehensive characterization of RAS mutations in colon and rectal cancers in old and young patients. *Nat Commun* 2019; 10: 3722.

169. Hunter JC, Manandhar A, Carrasco MA, *et al.* Biochemical and structural analysis of common cancer-associated KRAS mutations. *Mol Cancer Res* 2015; 13: 1325–1335.

170. Dienstmann R, Connor K, Byrne AT, *et al.* Precision therapy in RAS mutant colorectal cancer. *Gastroenterology* 2020; 158: 806–811.

171. Ostrem JM, Peters U, Sos ML, *et al.* K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. *Nature* 2013; 503: 548–551.

172. Hong DS, Fakih MG, Strickler JH, *et al.* KRAS G12C inhibition with sotorasib in advanced solid tumors. *New Engl J Med* 2020; 383: 1207–1217.

173. Johnson ML, Ou SHI, Barve M, *et al.* KRYSTAL-1: activity and safety of adagrasib (MRTX849) in patients with colorectal cancer (CRC) and other solid tumors harboring a KRAS G12C mutation. *Eur J Cancer* 2020; 138: S2.

174. Weiss J, Yaeger RD, Johnson ML, *et al.* LBA6 KRYSTAL-1: adagrasib (MRTX849) as monotherapy or combined with cetuximab (Cetux) in patients (Pts) with colorectal cancer (CRC) harboring a KRASG12C mutation. *Ann Oncol* 2021; 32: S1294.

175. Canon J, Rex K, Saiki AY, *et al.* The clinical KRAS(G12C) inhibitor AMG 510 drives anti-tumour immunity. *Nature* 2019; 575: 217–223.

176. Bar-Sagi D, Knelson EH and Sequist LV. A bright future for KRAS inhibitors. *Nat Cancer* 2020; 1: 25–27.

177. Maurer T, Garrenton LS, Oh A, *et al.* Small-molecule ligands bind to a distinct pocket in Ras and inhibit SOS-mediated nucleotide exchange activity. *Proc Natl Acad Sci USA* 2012; 109: 5299–5304.

178. Sun Q, Burke JP, Phan J, *et al.* Discovery of Small Molecules that Bind to K-Ras and Inhibit Sos-Mediated Activation. *Angew Chem Int Ed* 2012; 51: 6140–6143.

179. Kessler D, Gmachl M, Mantoulidis A, *et al.* Drugging an undruggable pocket on KRAS. *Proc National Acad Sci USA* 2019; 116: 15823–15829.

180. Hillig RC, Sautier B, Schroeder J, *et al.* Discovery of potent SOS1 inhibitors that block RAS activation via disruption of the RAS-SOS1 interaction. *Proc Natl Acad Sci USA* 2019; 116: 2551–2560.

181. Beck H, Nähse-Kumpf V, Larsen MS, *et al.* Cyclin-dependent kinase suppression by WEE1 kinase protects the genome through control of replication initiation and nucleotide consumption. *Mol Cell Biol* 2012; 32: 4226–4236.

182. Golan T, Hammel P, Reni M, *et al.* Maintenance olaparib for germline BRCA-mutated metastatic pancreatic cancer. *New Engl J Med* 2019; 381: 317–327.

183. Reilly NM, Novara L, Di Nicolantonio F, *et al.* Exploiting DNA repair defects in colorectal cancer. *Mol Oncol* 2019; 13: 681–700.

184. Rheinbay E, Nielsen MM, Abascal F, *et al.* Analyses of non-coding somatic drivers in 2,658 cancer whole genomes. *Nature* 2020; 578: 102–111.

185. Robinson DR, Wu Y-M, Lonigro RJ, *et al.* Integrative clinical genomics of metastatic cancer. *Nature* 2017; 548: 297–303.

186. Colwell J. NCI-MATCH trial draws strong interest. *Cancer Discov* 2016; 6: 334.

187. Massard C, Michiels S, Ferté C, *et al.* High-throughput genomics and clinical outcome in hard-to-treat advanced cancers: results of the MOSCATO 01 trial. *Cancer Discov* 2017; 7: 586–595.

188. Bertucci F, Gonçalves A, Guille A, *et al.* Prospective high-throughput genome profiling of advanced cancers: results of the PERMED-01 clinical trial. *Genome Med* 2021; 13: 87.

189. Hoff DDV, Stephenson JJ Jr, Rosen P, *et al.* Pilot Study Using Molecular Profiling of Patients' Tumors to Find Potential Targets and Select Treatments for Their Refractory Cancers. *J Clin Oncol* 2010; 28: 4877–4883.

190. Tsimberidou A-M, Iskander NG, Hong DS, *et al.* Personalized medicine in a phase I clinical trials program: the MD Anderson cancer center initiative. *Clin Cancer Res* 2012; 18: 6373–6383.

191. Trédan O, Wang Q, Pissaloux D, *et al.* Molecular screening program to select molecular-based recommended therapies for metastatic cancer patients: analysis from the ProfiLER trial. *Ann Oncol* 2019; 30: 757–765.

192. Horak P, Heining C, Kreutzfeldt S, *et al.* Comprehensive Genomic and Transcriptomic Analysis for Guiding Therapeutic Decisions in Patients with Rare Cancers. *Cancer Discov* 2021; 11: 2780–2795

193. Cocco E, Scaltriti M and Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. *Nat Rev Clin Oncol* 2018; 15: 731–747.

194. Drilon A, Laetsch TW, Kummar S, *et al.* Efficacy of Larotrectinib in TRK Fusion–Positive Cancers in Adults and Children. *N Engl J Med* 2018; 378: 731–739.

195. Doebele RC, Drilon A, Paz-Ares L, *et al.* 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.

196. Budinska E, Popovici V, Tejpar S, *et al.* Gene expression patterns unveil a new level of molecular heterogeneity in colorectal cancer. *J Pathol* 2013; 231: 63–76.

197. Schlicker A, Beran G, Chresta CM, *et al.* Subtypes of primary colorectal tumors correlate with response to targeted treatment in colorectal cell lines. *BMC Med Genomics* 2012; 5: 66.

198. Sadanandam A, Lyssiotis CA, Homicsko K, *et al.* A colorectal cancer classification system that associates cellular phenotype and responses to therapy. *Nat Med* 2013; 19: 619–625.

199. De Sousa E Melo F, Wang X, Jansen M, *et al.* Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013; 19: 614–618.

200. Marisa L, de Reyniès A, Duval A, *et al.* Gene expression classification of colon cancer into molecular subtypes: characterization, validation, and prognostic value. *PLOS Med* 2013; 10: e1001453.

201. Villamil BP, Lopez AR, Prieto SH, *et al.* Colon cancer molecular subtypes identified by expression profiling and associatedto stroma, mucinous type and different clinical behavior. *BMC Cancer* 2012; 12: 260.

202. Guinney J, Dienstmann R, Wang X, *et al.* The consensus molecular subtypes of colorectal cancer. *Nat Med* 2015; 21: 1350–1356.

203. Nguyen LH, Goel A and Chung DC. Pathways of colorectal carcinogenesis. *Gastroenterology* 2020; 158: 291–302.

204. Lenz H-J, Ou F-S, Venook AP, *et al.* Impact of consensus molecular subtype on survival in patients with metastatic colorectal cancer: results from CALGB/SWOG 80405 (alliance). *J Clin Oncol* 2019; 37: 1876–1885.

205. Stintzing S, Wirapati P, Lenz H-J, *et al.* Consensus molecular subgroups (CMS) of colorectal cancer (CRC) and 1st-line efficacy of FOLFIRI plus cetuximab or bevacizumab in the FIRE3 (AIO KRK-0306) trial. *Ann Oncol* 2019; 30: 1796–1803.

206. Mooi JK, Wirapati P, Asher R, *et al.* The prognostic impact of consensus molecular subtypes (CMS) and its predictive effects for bevacizumab benefit in metastatic colorectal cancer: molecular analysis of the AGITG MAX clinical trial. *Ann Oncol* 2018; 29: 2240–2246.

207. Okita A, Takahashi S, Ouchi K, *et al.* Consensus molecular subtypes classification of colorectal cancer as a predictive factor for chemotherapeutic efficacy against metastatic colorectal cancer. *Oncotarget* 2018; 9: 18698–18711.

208. Aderka D, Stintzing S and Heinemann V. Explaining the unexplainable: discrepancies in results from the CALGB/SWOG 80405 and FIRE-3 studies. *Lancet Oncol* 2019; 20: e274–e283.

209. Smeets D, Miller IS, O'Connor DP, *et al.* Copy number load predicts outcome of metastatic colorectal cancer patients receiving bevacizumab combination therapy. *Nat Commun* 2018; 9: 4112.

210. Sveen A, Bruun J, Eide PW, *et al.* Colorectal cancer Consensus molecular subtypes translated to preclinical models uncover potentially targetable cancer-cell dependencies. *Clin Cancer Res* 2018; 24: 794–806.

211. Linnekamp JF, van Hooff SR, Prasetyanti PR, *et al.* Consensus molecular subtypes of colorectal cancer are recapitulated in in vitro and in vivo models. *Cell Death Differ* 2018; 25: 616–633.

212. Fichtner M, Bozkurt E, Salvucci M, *et al.* Molecular subtype-specific responses of colon cancer cells to the SMAC mimetic Birinapant. *Cell Death Dis* 2020; 11: 1020.

213. Zhan T, Faehling V, Rauscher B, *et al.* Multi-omics integration identifies a selective vulnerability of colorectal cancer subtypes to YM155. *Int J Cancer* 2021; 148: 1948–1963.

214. Letai A. Functional precision cancer medicine – moving beyond pure genomics. *Nat Med* 2017; 23: 1028–1035.

215. Hidalgo M, Amant F, Biankin AV, *et al.* Patient-derived xenograft models: an emerging platform for translational cancer research. *Cancer Discov* 2014; 4: 998–1013.

216. Byrne AT, Alférez DG, Amant F, *et al.* Interrogating open issues in cancer precision medicine with patient-derived xenografts. *Nat Rev Cancer* 2017; 17: 254–268.

217. Kim MP, Evans DB, Wang H, *et al.* Generation of orthotopic and heterotopic human pancreatic cancer xenografts in immunodeficient mice. *Nat Protoc* 2009; 4: 1670–1680.

218. Bhimani J, Ball K and Stebbing J. Patient-derived xenograft models – the future of personalised cancer treatment. *Br J Cancer* 2020; 122: 601–602.

219. Hidalgo M, Bruckheimer E, Rajeshkumar NV, *et al.* A pilot clinical study of treatment guided by personalized tumorgrafts in patients with advanced cancer. *Mol Cancer Ther* 2011; 10: 1311–1316.

220. Sato T and Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. *Science* 2013; 340: 1190–1194.

221. Sato T, Stange DE, Ferrante M, *et al.* Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* 2011; 141: 1762–1772.

222. Sato T, Vries RG, Snippert HJ, *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* 2009; 459: 262–265.

223. Weeber F, Wetering M, van de Hoogstraat M, *et al.* Preserved genetic diversity in organoids cultured from biopsies of human colorectal cancer metastases. *Proc National Acad Sci USA* 2015; 112: 13308–13311.

224. Fujii M, Shimokawa M, Date S, *et al.* A colorectal tumor organoid library demonstrates progressive loss of niche factor requirements during tumorigenesis. *Cell Stem Cell* 2016; 18: 827–838.

225. van de Wetering M, Francies HE, Francis JM, *et al.* Prospective derivation of a living organoid biobank of colorectal cancer patients. *Cell* 2015; 161: 933–945.

226. Ooft SN, Weeber F, Dijkstra KK, *et al.* Patient-derived organoids can predict response to chemotherapy in metastatic colorectal cancer patients. *Sci Transl Med* 2019; 11: eaay2574.

227. Wensink GE, Elias SG, Mullenders J, *et al.* Patient-derived organoids as a predictive biomarker for treatment response in cancer patients. *Npj Precis Oncol* 2021; 5: 30

228. Vlachogiannis G, Hedayat S, Vatsiou A, *et al.* Patient-derived organoids model treatment response of metastatic gastrointestinal cancers. *Science* 2018; 359: 920–926.

229. Schütte M, Risch T, Abdavi-Azar N, *et al.* Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. *Nat Commun* 2017; 8: 14262.

230. Betge J, Rindtorff N, Sauer J, *et al.* Multiparametric phenotyping of compound effects on patient derived organoids. *Biorxiv* 2019; 2019: 660993

231. Ooft SN, Weeber F, Schipper L, *et al.* Prospective experimental treatment of colorectal cancer patients based on organoid drug responses. *ESMO Open* 2021; 6: 100103.