Clinical management of metastatic colorectal cancer in the era of precision medicine

Fortunato Ciardiello, MD, PhD [1]; Davide Ciardiello, MD[1,2]; Giulia Martini, MD, PhD[1]; Stefania Napolitano, MD, PhD[1]; Josep Tabernero, MD, PhD[3,4,5]; Andres Cervantes, MD, PhD[6,7]

[1]Division of Medical Oncology, Department of Precision Medicine, University of Campania Luigi Vanvitelli, Naples, Italy; [2]Division of Medical Oncology, IRCCS Foundation Home for the Relief of Suffering, San Giovanni Rotondo, Italy; [3]Medical Oncology Department, Vall d'Hebron Hospital Campus, Barcelona, Spain; [4]Institute of Oncology, University of Vic/Central University of Catalonia, Barcelona, Spain; [5]Oncology Institute of Barcelona-Quironsalud, Biomedical Research Center in Cancer, Barcelona, Spain; [6]Medical Oncology Department, Instituto de Investigación Sanitaria Valencia Biomedical Research Institute, University of Valencia, Valencia Spain; [7]Carlos III Institute of Health, Biomedical Research Center in Cancer, Madrid, Spain.

Corresponding Author: Fortunato Ciardiello, MD, PhD, Division of Medical Oncology, Department of Precision Medicine, Universita degli Studi della Campania Luigi Vanvitelli, Via S. Pansini 5, 80131 Naples, Italy (fortunato.ciardiello@unicampania.it).

DISCLOSURES: Fortunato Ciardiello was supported by a grant from Regione Campania (I-Cure Research Project Cup 21C17000030007). Andres Cervantes was supported by grants from the Instituto de Salud Carlos III (PI18/01909 and PI21/00689). Fortunato Ciardiello reports institutional research grants from Amgen, Merck KGaA, Merck Sharp & Dohme, Pfizer, Pierre Fabre, Roche, and Servier; and service on advisory boards for Bayer, Merck KGaA, Merck Sharp & Dohme, Pierre Fabre, Roche, and Servier outside the submitted work. Davide Ciardiello reports a travel grant from Sanofi outside the submitted work. Stefania Napolitano reports honoraria from Bristol Myers Squibb and Novartis outside the submitted work. Josep Tabernero reports advisory board or scientific consultancy fees from Array Biopharma, AstraZeneca, Avvinity, Bayer, Boehringer Ingelheim, Chugai, Daiichi-Sankyo, Roche, Genentech, HalioDX SAS, Hutchison MediPharma International, Ikena Oncology, Inspirna Inc, IQVIA, Lilly, Menarini, Merck KGaA, Merus, Merck Sharp & Dohme, Mirati, Neophore, Novartis, Ona Therapeutics, Orion Biotechnology, Peptomyc, Pfizer, Pierre Fabre, Samsung Bioepis, Sanofi, Seattle Genetics, Scandion Oncology, Servier, SotioBiotech, Taiho, Tessa Therapeutics, and TheraMyc outside the submitted work. Andres Cervantes reports institutional research grants from Genentech, Merck KGaA, Bristol Myers Squibb, Merck Sharp

Abstract: Colorectal cancer (CRC) represents approximately 10% of all cancers and is the second most common cause of cancer deaths. Initial clinical presentation as metastatic CRC (mCRC) occurs in approximately 20% of patients. Moreover, up to 50% of patients with localized disease eventually develop metastases. Appropriate clinical management of these patients is still a challenging medical issue. Major efforts have been made to unveil the molecular landscape of mCRC. This has resulted in the identification of several *druggable* tumor molecular targets with the aim of developing personalized treatments for each patient. This review summarizes the improvements in the clinical management of patients with mCRC in the emerging era of precision medicine. In fact, molecular stratification, on which the current treatment algorithm for mCRC is based, although it does not completely represent the complexity of this disease, has been the first significant step toward clinically informative genetic profiling for implementing more effective therapeutic approaches. This has resulted in a clinically relevant increase in mCRC disease control and patient survival. The next steps in the clinical management of mCRC will be to integrate the comprehensive knowledge of tumor gene alterations, of tumor and microenvironment gene and protein expression profiling, of host immune competence as well as the application of the resulting dynamic changes to a precision medicine-based continuum of care for each patient. This approach could result in the identification of individual prognostic and predictive parameters, which could help the clinician in choosing the most appropriate therapeutic program(s) throughout the entire disease journey for each patient with mCRC. **CA Cancer J Clin. 2022;72:000-000.**

Keywords: immunotherapy, metastatic colorectal cancer, molecular target therapy, precision medicine, tumor molecular profiling

Introduction

Colorectal cancer (CRC) is the third most common cancer in males and the second most common in females, with approximately 1.9 million new cases and 0.9 million deaths in 2020 worldwide.[1] CRC incidence has increased in recent years. It represents approximately 10% of all cancers and is the second most frequent cause of cancer deaths.[2,3] Therefore, CRC is a global public health challenge in terms of morbidity, mortality, and utilization of health care services, including increasingly high medical costs.[4]

Approximately 20% of patients with CRC have metastases at the time of diagnosis, whereas up to 50% of patients with initially localized disease will develop metastases. CRC can spread by lymphatic and hematogenous dissemination as well as by contiguous and peritoneal routes.[5] The most frequent metastatic sites are regional lymph nodes, liver, lungs, and peritoneum. The prognosis of patients who have metastatic CRC (mCRC) has significantly improved in the past 20 years with the introduction of more effective therapeutic approaches, including surgery of liver and lung metastases and novel anticancer drugs.[6] However, mCRC in most cases remains a noncurable disease.

This review summarizes the improvement in the clinical management of patients with mCRC in the emerging era of precision medicine. An individual, personalized therapeutic approach based on comprehensive knowledge of the complex genetic and environmental bases of the disease is required to achieve long-term control of mCRC with significantly better quality and quantity of life.

Molecular Pathogenic Routes of Colorectal Cancer Development

CRC is a heterogenous disease characterized by a plethora of molecular alterations that determine the dysregulation of different signaling pathways, leading to tumor onset, progression, and invasiveness.[7] The high intertumor and intratumor variability at different genetic levels highlights the complex molecular biology of the neoplasm, which, in turn, affects tumor response to therapies and patient survival.[8] Even in the presence of a recognized and *druggable* gene alteration, the antitumor activity of the corresponding matched target therapy remains unpredictable.[9] Hence, better knowledge of the multiple developmental trajectories of CRC is fundamental to identify and tackle cancer vulnerabilities and to change the clinical course of the disease.[10]

Molecular heterogeneity may already be found at premalignant stages. This is determined by genetic and epigenetic regulations, which will influence different CRC molecular profiles.[8] In this regard, 3 distinct pathways have been identified in the pathogenesis of CRC: chromosomal instability, high microsatellite instability (MSI-H), and CpG island methylator phenotype (CIMP).[11]

Chromosomal instability is reported in approximately 65% to 70% of sporadic CRC and refers to an abnormal accumulation of gain or loss of entire regions of chromosomes that, in turn, results in chromosome number alterations, chromosome amplifications, and/or loss of heterozygosity (LOH).[12] In 1990, Fearon and Volgestein described a multistep genetic model for the adenoma-to-carcinoma sequence that represented a milestone in the understanding of CRC initiation and progression.[13] The first step in the tumorigenic process is silencing, mutation, or LOH of the gatekeeper adenomatous polyposis coli (*APC*) gene. Loss of APC function causes the constitutive activation of WNT signaling, which controls the proliferation, differentiation, and renewal of intestinal stem cells, leading to the transformation of normal epithelium to early adenoma. A subsequent accumulation of gene alterations, including *KRAS*, *TP53*, and LOH 18q, occurs during the transition from adenoma to carcinoma. Finally, dysregulation of the phosphoinositide-3 kinase (PI3K) and transforming growth factor β (TGF-β) pathways is frequently observed in later stages.[14,15]

The second group of CRCs (approximately 15%-20%) is characterized by a deficit in the DNA mismatch-repair (dMMR) system, leading to an abnormal accumulation of gene mutations.[16] The integrity of the dMMR machinery (MLH1, MSH2, MSH6, and PMS2 proteins) is necessary for recognizing, correcting, and thus preventing errors during DNA replication.[17] A dysfunctional dMMR system causes the generation of novel microsatellite fragments and of neoantigens, thus determining the MSI-H phenotype. Germline mutations in one of the dMMR genes are the hallmark of hereditary nonpolyposis CRC or Lynch syndrome, in which there is an increased risk of developing different gastrointestinal malignancies, including CRC.[18,19]

The serrated neoplasia pathway is the third pathogenic route in CRC development.[20] Epigenomic instability of serrated neoplasia relies on the dysregulation of CpG island methylation. Aberrant hypermethylation in the promoter region of suppressor genes results in gene silencing and favors tumor onset (CIMP phenotype).[21] Of note, nonhereditary, sporadic MSI-H tumors also could be the result of *MLH1* silencing and are strictly associated with *BRAF* valine-to-glutamate substitution at codon 600 (V600E) mutations.[22] Epigenetic silencing of other tumor suppressor genes, such as *MGMT*, *p16INK4*, and *IGFBP7*, could play a significant role in the development of CIMP CRC.[23-25] However, a subgroup of MSI-H tumors could also present features of serrated neoplasia.

Mutational Landscape of Metastatic Colorectal Cancer

Major efforts have been made to better understand the genomic landscape of CRC and to identify *druggable* mutations, with the aim of developing a truly precision medicine-based, personalized treatment for each patient.[26] The effective development of precision oncology for patients with mCRC has been more challenging than expected.[27-29] This may be explained in part by the genetic heterogeneity of these tumors, the paucity of *druggable* targets, and the complex interplay between different signaling pathways that could allow cancer cells to escape single-target inhibition.[9]

& Dohme, Roche, Beigene, Bayer, Servier, Lilly, Novartis, Takeda, Astellas, Takeda, and Fibrogen; and honoraria or speaker's fees from Amgen, Merck KGaA, Roche, Bayer, Servier, and Pierre Fabre outside the submitted work. Giulia Martini reports no conflicts of interest.

doi: 10.3322/caac.21728. Available online at cacancerjournal.com

After more than 20 years of translational and clinical investigation, targeting the epidermal growth factor receptor (EGFR) family and its intracellular signaling pathways still represents the most relevant keystone for the targeted molecular treatment of mCRC (Fig. 1).[30]

EGFR (HER1) is a growth factor receptor belonging to a family of cell membrane growth factor receptors with tyrosine kinase enzymatic activity, which includes HER2/*c-neu* (ERBB2), HER3 (ERBB3), and HER4 (ERBB4).[31] Numerous specific ligands that activate EGFR signaling have been discovered, including epidermal growth factor (EGF), transforming growth factor α (TGF-α), amphiregulin, and epiregulin. Ligand binding to the EGFR extracellular domain induces receptor tridimensional conformation changes, which allow receptor homodimerization or heterodimerization (with one of the other 3 EGFR-related receptors), which, in turn, triggers the phosphorylation of specific tyrosine residues in the EGFR intracellular domain. This activates a complex intracellular signaling cascade, including the RAS-RAF-MEK-MAPK and PTEN-I3K-AKT-mTOR pathways, which regulate cancer cell proliferation, survival, invasiveness, metastatic spread, and tumor-induced angiogenesis.[32]

The *RAS* family comprises 3 different genes that encode for 4 proteins: HRAS, NRAS, KRAS4A, and KRAS4B (the latter 2 are isoforms that are produced by different splicing).[33] Because KRAS4B is the predominant splice variant, it is also simply referred as KRAS. In physiologic conditions, RAS (KRAS and NRAS) changes continuously between the active guanosine triphosphate (GTP)-bound and the inactive guanosine diphosphate (GDP)-bound states, depending on the cellular context and on signaling stimuli. Transition from the stable, inactive GDP-bound form to the active GTP-bound form is stimulated by guanine nucleotide exchange-factors (GEFs), whereas the switch to the reverse process is mediated by GTPase-activating proteins (GAPs). Mutations that determine the constitutive activation of *RAS* in the GTP-bound state promote tumorigenesis and modulate the tumor microenvironment by inducing immune escape and cancer progression.[34] The presence of *KRAS*-activating mutations was the first predictive negative biomarker discovered for anti-EGFR treatment in mCRC.[35] Different studies

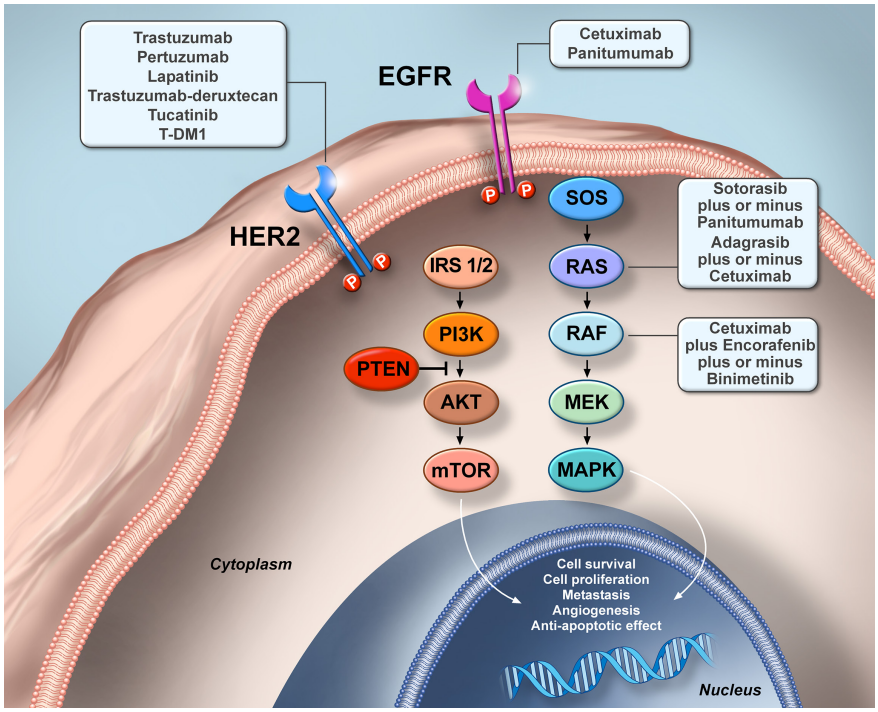

FIGURE 1. EGFR and HER2 Pathways in Metastatic Colorectal Cancer. Epidermal growth factor receptor (EGFR) is a member of the human epidermal growth factor receptor (HER)/erbB family, which includes EGFR (ERBB1), HER2/c-neu (ERBB2), HER3 (ERBB3), and HER4 (ERBB4). After ligand-binding receptor homodimerization or heterodimerization occurs, this activates 2 main intracellular downstream pathways (P): RAS-RAF-MEK-MAPK and PI3K-AKT-mTOR, which are involved in the regulation of cancer cell proliferation, survival, migration, invasion, and metastasis. Adagrasib is an irreversible inhibitor of *KRAS* glycine-to-cysteine substitution at codon 12 (G12C)-mutated protein; binimetinib, a reversible MEK inhibitor; cetuximab, a human-mouse chimeric immunoglobulin G1 (IgG1) anti-EGFR monoclonal antibody; encorafenib, a reversible inhibitor of BRAF substitution of valine by glutamate in codon 600 (V600E)-mutated protein; lapatinib, a reversible anti-EGFR and anti-HER2 tyrosine kinase inhibitor; panitumumab, a fully human IgG2κ anti-EGFR monoclonal antibody; pertuzumab, a humanized IgG1 anti-HER2 monoclonal antibody; sotorasib, an irreversible inhibitor of KRAS G12C-mutated protein; T-DM1, trastuzumab emtansine is an antibody-drug conjugate composed of DM1 (a microtubule inhibitor) covalently linked to trastuzumab; trastuzumab, a humanized IgG1 anti-HER2 monoclonal antibody; trastuzumab-deruxtecan, an antibody-drug conjugate composed of deruxtecan (a topoisomerase I inhibitor) covalently linked to trastuzumab; tucatinib, a reversible anti-HER2 tyrosine kinase inhibitor.

have demonstrated that *KRAS* exon 2 mutations lead to the constitutive activation of MAPK signaling, bypassing the upstream blockade of EGFR with the therapeutic monoclonal antibodies cetuximab or panitumumab.[36-38] Subsequent investigations have shown that not only *KRAS* exon 2 but also other *KRAS* and *NRAS* mutations confer resistance to anti-EGFR treatments.[39,40] More than 40% mCRCs have *KRAS* mutations, which are most frequent in exon 2 and in codons 12 (approximately 80% of all *KRAS* mutations) and 13 and are less common in exons 3 (codons 59 and 61) and 4 (codons 117 and 146).[41] *NRAS* mutations are rare (5%-10% of mCRC) and occur mostly in exons 3 (codon 61) and 2 (codons 12 and 13).[42] *RAS* mutations are generally associated with a poor prognosis and an impaired response to anticancer therapies. However, not all *RAS* mutations have the same impact on the clinical course of the disease. The *KRAS* substitution of glycine by cysteine within codon 12 (G12C) is reported in approximately 3% to 4% of mCRC and correlates with major aggressiveness and worse outcome.[43,44]

BRAF mutations are observed in 10% to 15% of mCRC.[45,46] The substitution of valine by glutamate within codon 600 (V600E) is the most frequent *BRAF* alteration. This mutation is almost mutually exclusive with *KRAS* and *NRAS* mutations. It causes constitutive activation of the MAPK signaling pathway, confers high clinical aggressiveness and resistance to anti-EGFR monoclonal antibody treatment, and is associated with poor survival.[47] *BRAF* V600E-mutant mCRC may display the CIMP phenotype and, in 10% to 20% of cases, is also MSI-H.[48,49] *BRAF* V600E-mutant mCRC more often correlates with older age, female sex, proximal colon (right side location), peritoneal and hepatic metastatic spread, and mucinous or poorly differentiated histology.[50] Intriguingly, *BRAF* V600E-mutant CRC is not a unique disease and could display various responses to molecular target therapies.[51] Two subtypes of *BRAF* V600E mutants have been identified: BM1 and BM2, which are distinguished by different gene expression profiles.[52] The BM1 subtype occurs in one-third of cases; it is characterized by KRAS and AKT pathway activation and a strong immune infiltrate, increased angiogenesis, TGF-β dysregulation, and epithelial-to-mesenchymal transition (EMT). The BM2 group represents two-thirds of *BRAF* V600E mCRC; it is enriched in activated metabolic pathways and has high CDK1 and low cyclin-D1 levels. Of note, a trend toward poorer survival has been observed in the BM1 *BRAF* V600E subtype compared with the BM2 subtype. Non-V600E *BRAF*-mutant mCRC is rare, occurring in approximately 2% of cases.[53] A large, multicenter, retrospective study has recently shown that overall survival (OS) is significantly longer in patients who have non-V600E *BRAF*-mutant mCRC compared with those who have either *BRAF* V600E-mutant or *BRAF* wild-type (WT) mCRC.[53]

Regulatory agencies, including the US Food and Drug Administration (FDA) and the European Medicine Agency, require mandatory hot-spot mutation analysis for *KRAS*, *NRAS*, and *BRAF* before treatment choices for patients with mCRC. In fact, the use of anti-EGFR monoclonal antibodies (cetuximab or panitumumab) is restricted to *RAS* WT tumors because EGFR inhibition has therapeutic efficacy only in mCRC in which the EGFR-driven intracellular signaling machinery is composed of normal, nonmutated proteins.[54,55] Assessment of the mutational status of *KRAS*, *NRAS*, and *BRAF* may be done using real time-polymerase chain reaction or next-generation sequencing (NGS) on DNA extracted either from the primary tumor or from a metastatic site.[56] Recent studies also support the use of liquid biopsy-based approaches for detecting the presence of circulating tumor DNA (ctDNA) in the plasma of patients with mCRC.[57] Relatively good concordance in the detection of *RAS* and *BRAF* mutations in tumor tissues compared with plasma has been reported.[58] A potential advantage of liquid biopsy is the possibility of repeating this procedure to evaluate the dynamic tumor mutation changes under the selective pressure of therapies for a better choice of molecular target drugs to use.[59] Furthermore, assessment of ctDNA allows the comprehensive detection of different mutated cancer clones, which may arise in different metastatic sites.[60] In this respect, several NGS extended gene panels for liquid biopsy analysis are currently available.

HER2 gene alterations are relatively rare in mCRC. HER2 protein overexpression due to *HER2* gene amplification has been reported in approximately 3% of mCRCs, mostly in patients with *RAS/BRAF* WT tumors.[61] HER2 overexpression generally causes primary resistance to anti-EGFR therapies, as suggested by retrospective studies.[62-64] However, a subset of patients with *HER2*-amplified mCRC might still benefit from EGFR inhibition.[65]

Other rare gene alterations include receptor tyrosine kinase (RTK) fusion genes, such as *ALK*, *ROS1*, and *NTRK*, occurring in 0.2% to 2.4% of mCRCs.[66] Of note, these RTK gene rearrangements are more frequent in patients with right-sided primary CRC, *RAS/BRAF* WT tumors, and MSI-H tumors. In a retrospective study, poorer survival was observed in patients who had mCRC with *ALK*, *ROS1*, or *NTRK* gene rearrangements. Primary resistance to anti-EGFR therapies was also suggested in these cases.

The presence of MSI-H or dMMR has been identified in approximately 15% of CRCs, with some differences according to tumor stage. Frequency is higher in localized CRC compared with mCRC: approximately 20% in stage II tumors, 12% in stage III tumors, and 5% in stage IV tumors.[17] Approximately 3% of MSI-H tumors are associated with the hereditary Lynch syndrome, whereas another 12% are caused by sporadic, acquired hypermethylation of the *MLH1*

gene promoter, which occurs in tumors with the CIMP phenotype. In any case, the presence of impaired mismatch-repair function causes an accumulation of DNA replication errors, facilitates insertions or deletions, and leads to the generation of a huge number of neoantigens.[67] Some of these neoantigens will be processed, presented on the major histocompatibility complex (MHC), and recognized as foreign molecules by T cells. This will determine high levels of tumor-infiltrating lymphocytes (TILs) in MSI-H/dMMR mCRC. However, the overexpression of inhibitory immune checkpoint molecules could suppress activation of the immune system in these cases.[68] Therefore, removing the brake with the use of antiprogrammed death 1 (anti–PD-1)/programmed death ligand 1 (PD-L1) monoclonal antibodies could activate the immune response, with strong antitumor activity.[69] Therefore, because immunotherapy with immune checkpoint inhibitors could be highly effective in these patients, an evaluation of tumor microsatellite status is highly recommended for each patient with mCRC.[55] Diagnosis of MSI-H/dMMR may be performed using different methodologies, including real time-polymerase chain reaction analysis, immunohistochemistry, or NGS, with a relatively good level of concordance.[69]

High tumor mutational burden is not exclusively observed in MSI-H/dMMR tumors. In fact, DNA polymerase ε (POLE) and DNA polymerase δ (POLD1) are 2 enzymes involved in DNA synthesis and repair.[70] Mutations of these genes affect DNA repair and lead to the production of high numbers of neoantigens, rendering patients with mCRC whose tumors harbor such mutations potentially responsive to immune checkpoint inhibitors.[71] *POLE* mutations are rare, being detected in approximately 1% to 2% of mismatch-repair–proficient (pMMR) mCRC, with higher prevalence in younger patients.

Consensus Molecular Subtype Classification of Colorectal Cancer

Major efforts have been dedicated to identifying biologically homogeneous CRC subtypes for a better prognostic assessment and for improving the development of novel therapies.[72] An international consortium performed a comprehensive transcriptome analysis of gene expression on tumor cells as well as on tumor-infiltrating stroma and the tumor microenvironment. Four different consensus molecular subtypes (CMS) groups were defined.[73] CMS1 tumors (MSI-H immune; approximately 14% of early stage CRCs) are hypermutated and hypermethylated, display an enrichment in *BRAF* V600E mutations, and show robust immune infiltration (CD8-positive T cells, TILs, and CD68-positive macrophages). CMS2 tumors (canonical; approximately 37% of early stage CRCs) are epithelial tumors characterized by activation of the WNT and MYC pathways. CMS3 tumors (metabolic; approximately 13% of early stage CRCs) frequently have *KRAS* mutations, display deregulated cancer cell metabolic pathways, and have lower gene copy number alterations. CMS4 tumors (mesenchymal; approximately 23% of early stage CRCs) are characterized by the activation of pathways related to EMT, such as TGF-β signaling, enhanced angiogenesis, stromal activation, and inflammatory infiltrate.

The CMS groups in part may recapitulate the divergent pathogenic routes of adenoma-to-carcinoma progression. Interestingly, serrated sessile adenomas, which depend on the levels of TGF-β, may progress to CMS4 (with high TGF-β signaling) and to CMS1 (with low TGF-β signaling) tumors.[72,74] Remarkably, adenomatous polyps displayed a CMS2-like phenotype with prominent WNT and MYC activation, whereas hyperplastic and serrated polyps with a CMS1-like phenotype displayed a significant enrichment of genes involved in immune and stromal infiltration.[75] Only a limited number of adenomas with a CMS4-like phenotype were included in the analysis. However, a significant enrichment for stromal signatures along with TGF-β activation was observed.

Distinct characteristics and outcomes have been reported across the different CMS groups.[73] CMS1 tumors are more frequent in female patients with right-sided primary tumors, have undifferentiated histopathologic grading, and exhibit a poor outcome after relapse. CMS2 tumors are more frequently localized in the left side of the colon or in the rectum and have a better prognosis. CMS4 tumors are more often diagnosed in advanced-disease stage with poorer relapse-free survival and OS. Recently, a translational study that included more than 1700 primary tumor samples from patients treated on the PETACC-8 adjuvant trial (ClinicalTrials.gov identifier NCT03362684), reported that, in 55% of tumor samples, ≥2 CMS groups could be identified. These results strongly suggest that intratumor CMS heterogeneity is a frequent finding.[76] Of note, intratumor heterogeneity was correlated with reduced disease-free survival and with worse OS. Another layer of complexity could be represented by the presence of intrapatient tumor heterogeneity.[77] In this regard, an analysis of the transcriptomic profiles from 317 primary tumors and 295 liver metastases indicated that metastases were classified more frequently as CMS2 or CMS4 and less frequently as CMS1 or CMS3 compared with primary tumors. This heterogeneity may be a consequence of the complex tumor biology and of the interaction between cancer cells and the microenvironment. In fact, CMS classification could be affected by gene expression changes from the tumor core to the invasion front, which may be caused by regional differences in immune infiltrates and stroma content and composition.[78]

Integrating Molecular Target Therapies in the Continuum of Care of Metastatic Colorectal Cancer

Major therapeutic advances have been achieved in mCRC, resulting in clinically relevant survival improvements.[2,79,80] The chemotherapy era started with the development of 5-fluorouracil (5-FU), a fluoropyrimidine antimetabolite drug, in 1957.[81,82] Key developments in the early 2000s included the addition of irinotecan, a topoisomerase I inhibitor, and oxaliplatin, a DNA cross-linking drug, as components of 5-FU–based cytotoxic combination therapies (FOLFIRI [folinic acid, 5-FU, and irinotecan] and FOLFOX [folinic acid, 5-FU, and oxaliplatin], respectively). These combinations provide higher response rates and better progression-free survival (PFS) than 5-FU alone. Furthermore, the possibility of sequential treatments with FOLFIRI and FOLFOX, or vice versa, has established 2 subsequent lines of therapy for most patients with mCRC.[83,84] This has led to a significant increase in median survival from 6 to 9 months up to 15 to 18 months.

A major improvement with more effective therapeutic options has been the introduction of molecular target drugs (initially, antivascular endothelial growth factor-A [anti–VEGF-A], VEGF-A, and anti-EGFR monoclonal antibodies) in addition to chemotherapy.[85] The increasing number of effective anticancer drugs (Fig. 2), together with the improvement of surgical procedures and the availability of local ablative treatments for liver and lung metastases, has significantly increased patient survival by allowing a *continuum-of-care* treatment strategy with multiple lines of therapy (≥4 in most patients). Currently, most patients with mCRC experience a survival of 24 to 36 months. Furthermore, up to one-third of patients who have mCRC limited to the liver (and, in some cases, the lungs) may even be cured by an integrated clinical management with systemic medical treatments and locoregional radical surgical removal of metastases.[86,87]

The first successful step toward personalized cancer medicine has been the definition of different treatment options and sequences, which are based on tumor molecular stratification.[88] The search for *RAS* and *BRAF* gene alterations and the evaluation of microsatellite status are currently the gold standard for mCRC molecular characterization, which is needed for selecting the most appropriate treatments (Figs. 3 and 4).[55] Moreover, it is becoming standard of care to also screen for *HER2* gene amplification.[55] In addition to molecular stratification, several factors influence the choice of up-front treatment, including patient characteristics (performance status, comorbidities, age) and tumor features (tumor burden, sites of metastases, the presence of potentially resectable liver or lung metastases, primary tumor location). Effective first-line treatment is a critical determinant for therapeutic efficacy.

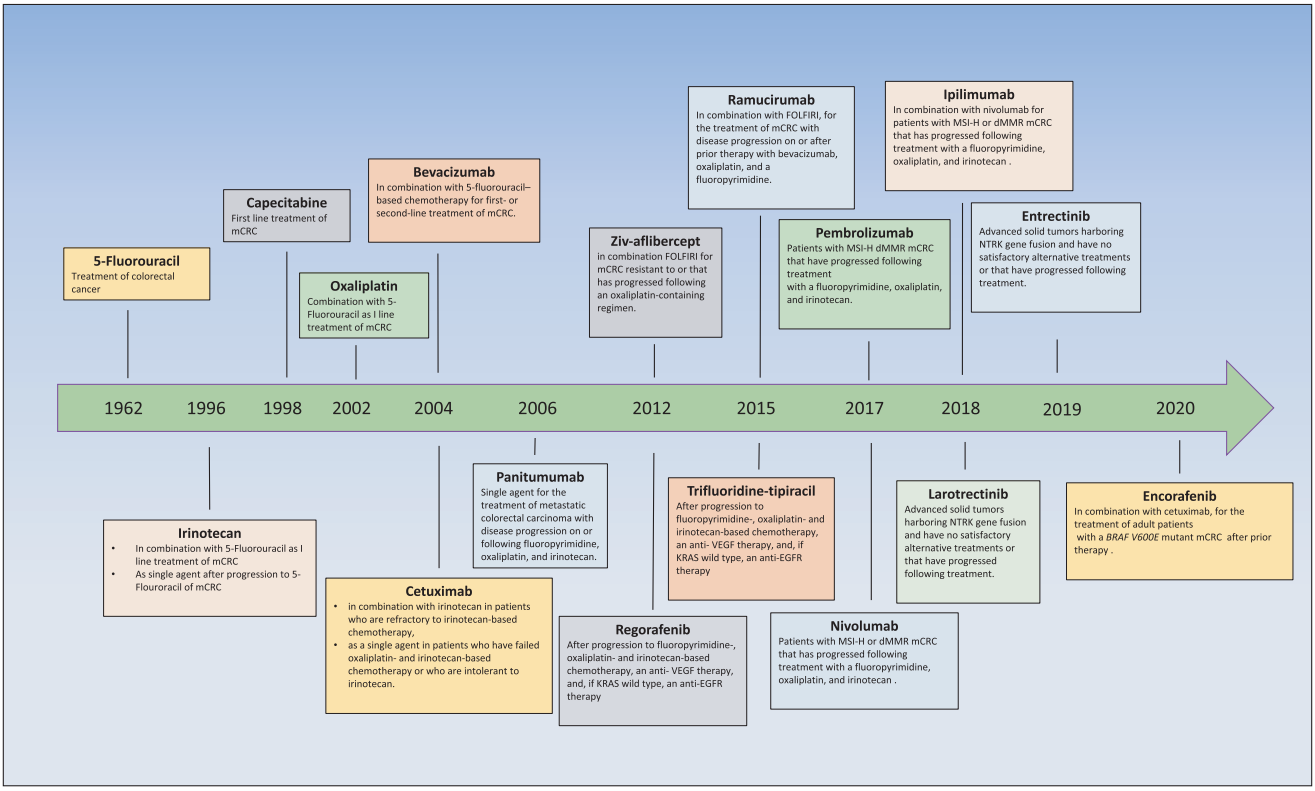

FIGURE 2. Timeline of US Food and Drug Administration Approvals With the First Indication for Each Drug in the Treatment of Metastatic Colorectal Cancer. dMMR indicates DNA mismatch-repair–deficient; FOLFIRI, folinic acid, 5-fluorouracil, and irinotecan; mCRC, metastatic colorectal cancer; MSI-H, high microsatellite instability.

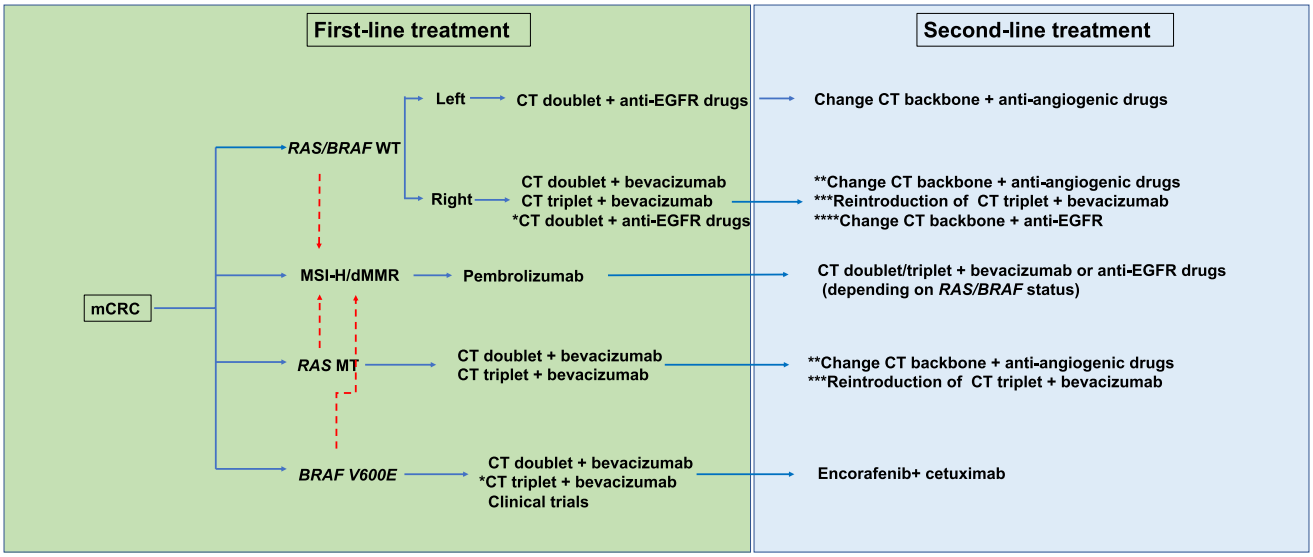

FIGURE 3. Therapeutic Algorithm of First-Line and Second-Line Treatments for "Fit" Patients With Unresectable Metastatic Colorectal Cancer. The algorithm indicates treatment according to molecular stratification for RAS (*KRAS* and *NRAS*), *BRAF* V600E (substitution of valine by glutamate in codon 600), high microsatellite instability (MSI-H)/DNA mismatch-repair–deficient (dMMR) genetic alterations *if the goal is tumor shrinkage. **In patients who obtain a benefit from chemotherapy (CT) plus bevacizumab in first-line treatment, bevacizumab also could be maintained in second-line treatment, and ziv-aflibercept or ramucirumab could be used in combination with FOLFIRI (folinic acid, 5-fluorouracil [5-FU], and irinotecan) after progression on prior oxaliplatin-based therapy plus bevacizumab. ***If a CT triplet plus bevacizumab was used in the first line for a fixed time, then treatment was deescalated to 5-FU plus bevacizumab. ****It may be an option not to use anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibodies in the first line. CT doublet indicates FOLFIRI or FOLFOX (folinic acid, 5-FU, and oxaliplatin); CT triplet, FOLFOXIRI (folinic acid, 5-FU, oxaliplatin, and irinotecan). The antiangiogenic drugs used were bevacizumab, ziv-aflibercept, and ramucirumab; and the anti-EGFR drugs were cetuximab and panitumumab. mCRC indicates metastatic colorectal cancer; MT, mutated; WT, wild type.

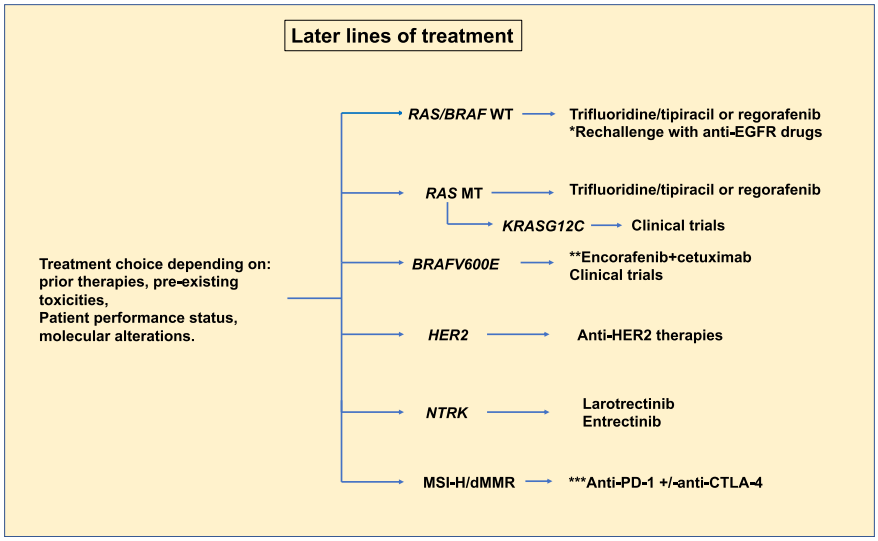

FIGURE 4. Therapeutic Algorithm of Later Lines of Treatment for Unresectable Metastatic Colorectal Cancer. *Although not yet considered as a standard of care (for the absence of randomized trials), rechallenge with epidermal growth factor receptor (EGFR) inhibitors has shown clinically relevant antitumor activity in patients with *RAS/BRAF* wild-type (WT) circulating tumor DNA, as assessed by pretreatment liquid biopsy. **If not administered in the previous line of treatment. ***In patients who have not received prior immunotherapy (anti–PD-1, nivolumab or pembrolizumab; anti–CTLA-4, ipilimumab). +/– indicates with or without; dMMR, DNA mismatch-repair–deficient; MSI-H, high microsatellite instability; MT, mutated.

The most appropriate first-line chemotherapy options include FOLFIRI or FOLFOX.[84,85] A possibility for further increasing objective responses, PFS, and OS in selected *fit* patients with mCRC is to combine these chemotherapies in the *triplet* FOLFOXIRI regimen (folinic acid, 5-FU, oxaliplatin, and irinotecan).[89] Because the maximum clinical benefit is achieved during first-line treatment, strategies to consolidate antitumor response and to maintain disease control in a balance with safety and tolerability of treatments may vary. These strategies include the duration of first-line therapy, the use of *chemotherapy-free* intervals, and/or the implementation of less toxic, long-term *maintenance* therapies.

In this scenario, targeting neoangiogenesis has been the first biologic therapy with additive or synergistic activity

when combined with chemotherapy.[90-93] Tumor-induced angiogenesis is a hallmark of cancer development and progression in all solid cancers, including CRC. Bevacizumab, a humanized anti–VEGF-A monoclonal antibody, is the first antiangiogenic drug to be successfully added to the therapeutic armamentarium for mCRC. Several phase 2 and 3 studies have established the efficacy of adding bevacizumab to 5-FU–based chemotherapy regimens in improving PFS and OS in patients with mCRC, as shown in Table 1.[90-101] Bevacizumab can be used in either first-line or second-line therapy or, according to a *beyond progression* strategy, in both first-line and second-line therapy by switching chemotherapy.[97,98] Two other antiangiogenic drugs (ziv-aflibercept and ramucirumab) may be combined with FOLFIRI in the second line after progression on FOLFOX plus bevacizumab.[99-100] Furthermore, regorafenib, a broad-spectrum, antiangiogenic, multikinase inhibitor, can be used in third-line therapy after disease progression.[102,103] Unfortunately, despite 2 decades of extensive translational and clinical investigation, no predictive biomarkers of antitumor activity or efficacy of antiangiogenic drugs have been identified. Therefore, similar to chemotherapy, these drugs are still used in unselected patients with mCRC.

Conversely, the therapeutic use of the 2 anti-EGFR monoclonal antibodies cetuximab and panitumumab is restricted to selected patients with mCRC. However, this selection is based on predictive molecular biomarkers of lack of response (ie, *RAS*-activating or *BRAF*-activating mutations) rather than the presence of positive predictive biomarkers of antitumor activity and efficacy[104,105] (Tables 2 and 3).[39,40,106-115] Either cetuximab or panitumumab is combined with standard chemotherapy regimens as first-line or second-line therapy after chemotherapy plus bevacizumab for patients with *RAS/BRAF* WT tumors. Extensive clinical investigation has been done to evaluate which is the most effective first-line therapy in patients with *RAS/BRAF* WT mCRC. A meta-analysis of 6 randomized phase 2 and 3 clinical trials has evaluated the combination of chemotherapy plus cetuximab or panitumumab compared with the same chemotherapy with or without bevacizumab in patients with *RAS/BRAF* WT mCRC.[116] An anti-EGFR monoclonal antibody plus chemotherapy is the most effective treatment in terms of tumor response, PFS, and OS in patients with mCRC whose primary tumors are localized in the left side of the colon or in the rectum. In this respect, mCRC that originates in the right colon has different gene alterations and expression profiles, which determine reduced sensitivity to EGFR inhibition.

In addition to left-sided primary tumor localization, anti-EGFR monoclonal antibody treatment seems to be more effective in patients whose tumors express high levels of amphiregulin and epiregulin, which are identified more frequently in well differentiated epithelial mCRC.[31] Conversely, the features of EMT, including TGF-α overexpression, enhanced AXL and EPHA2 signaling, and increased TGF-β signaling, are associated with resistance to anti-EGFR drugs.[117-120]

The role of CMS classification in predicting response to chemotherapy combined with antiangiogenic or anti-EGFR drugs is controversial and is still debated.[121] Retrospective analyses of 2 large, randomized phase 3 trials, in which different chemotherapies (FOLFIRI or FOLFOX) in combination with bevacizumab or cetuximab have been evaluated as first-line treatment in patients with *RAS* WT mCRC, have been published, with discordant results. In fact, patient survival was better with bevacizumab plus chemotherapy in those with CMS1 and CMS4 tumors, whereas cetuximab plus chemotherapy determined better survival in those with CMS2 tumors, according to the Cancer and Leukemia Group B trial CALGB-80405 (ClinicalTrials.gov identifier NCT00265850), in which 75% of patients received FOLFOX and 25% received FOLFIRI. Conversely, in the FIRE3 trial (ClinicalTrials.gov identifier NCT00433927), in which 100% of patients were treated with FOLFIRI, a survival benefit in favor of cetuximab versus bevacizumab was reported for the CMS3 and CMS4 subgroups.[122,123] The discrepancies between the results of these 2 studies highlight the complexity of interactions between mCRC biology and treatment.[121] In fact, the chemotherapy backbone (FOLFIRI or FOLFOX) in combination with either cetuximab or bevacizumab may have different functional interactions with the tumor microenvironment, which may affect antitumor activity. It has been hypothesized that irinotecan may synergize with cetuximab in all CMS groups, whereas oxaliplatin may synergize with cetuximab only in fibroblast-poor tumor microenvironments (CMS2 and CMS3) and may antagonize with cetuximab in fibroblast-rich tumor microenvironments (CMS1 and CMS4). However, the CMS classification is not yet considered a valid predictive biomarker for choosing either antiangiogenic or anti-EGFR drugs.

Although chemotherapy combined with anti-EGFR monoclonal antibodies is highly effective in patients with mCRC who have left-sided *RAS/BRAF* WT tumors, with major objective responses observed in approximately two-thirds of patients and a median PFS of 11 months, disease progression occurs in all patients.[116] In approximately one-third of patients, this is caused by the emergence of *RAS*-mutant and, less frequently, *BRAF*-mutant or ectodomain *EGFR*-mutant cancer cell clones as escape mechanism(s) of EGFR inhibition.[124] In fact, treatment with anti-EGFR drugs eliminates *RAS/BRAF* WT-sensitive clones, whereas acquired *RAS*-mutant–resistant clones become the prevalent cancer cell population. Therefore, second-line treatment

TABLE 1. Key Phase 2 and 3 Trials of Antiangiogenic Drugs (Bevacizumab, Ziv-Aflibercept, and Ramucirumab) in Combination With Chemotherapy in First-Line or Second-Line Treatment of Patients With Metastatic Colorectal Cancer

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
Kabbinavar 2003[90]	2	36 vs 35 vs 33	First line	5-FU/LV plus bevacizumab (low or high dose) vs 5-FU/LV	40.0 (low-dose arm) vs 17.0	9.0 (low-dose arm) vs 5.2	21.5 (low-dose arm) vs 13.8	First study showing efficacy of bevacizumab plus 5-FU/LV (low-dose arm received 5 mg/kg)
Hurwitz 2004[91]	3	402 vs 411	First line	IFL plus bevacizumab vs IFL plus placebo		10.6 vs 6.2 ($P < .001$)	20.3 vs 15.6 ($P < .001$)	The first randomized phase 3 study demonstrating that bevacizumab added to IFL as first-line treatment improves PFS and OS
Saltz 2008[92]	3	699 vs 701	First line	FOLFOX/CAPOX plus bevacizumab vs FOLFOX/CAPOX plus placebo	47.0 vs 49.0 ($P = .31$; OR, 0.90)	9.4 vs 8.0 ($P = .0023$; HR, 0.83)	21.3 vs 19.9 ($P = .077$; HR, 0.89)	The addition of bevacizumab to oxaliplatin plus fluoropyrimidine-based first-line treatment improves PFS; no difference in terms of OS.
AIO-0207: Hegewisch-Becker 2015[94]	3	158 vs 156 vs 158	Maintenance after 5-FU/LV/oxaliplatin/bevacizumab first-line treatment	Fluoropyrimidine plus bevacizumab vs bevacizumab vs no treatment		6.3 vs 4.6 vs 3.5 vs ($P < .0001$)	20.2 vs 21.9 vs 23.1 ($P = .77$)	Primary end point (time to failure of strategy from randomization) shows noninferiority of bevacizumab compared with fluoropyrimidine plus bevacizumab but not for no treatment as maintenance strategy; however, secondary end point (PFS) suggests that fluoropyrimidine plus bevacizumab could be the maintenance treatment choice
CAIRO 3: Simkens 2015[95]	3	279 vs 279	Maintenance after 6 cycles of CAPOX/bevacizumab first-line treatment	Capecitabine plus bevacizumab vs observation		mPFS1: 8.5 vs 4.1 ($P < .0001$; HR, 0.43); mPFS2: 11.7 vs 8.5 ($P < .0001$; HR, 0.63)	21.6 vs 18.1 ($P = .22$; HR, 0.89)	Randomized phase 3 study demonstrating that maintenance treatment with capecitabine plus bevacizumab after 6 cycles of CAPOX plus bevacizumab, compared with observation, is effective, without compromising quality of life; *PFS1:* time from randomization until first progression after observation or maintenance treatment; *PFS2 (primary end point):* 1) time from randomization until second progression after observation or maintenance (for those who had a first progression) while under treatment with reintroduction of CAPOX plus bevacizumab, 2) date of first progression while under maintenance or observation, and 3) end of trial (for those without second progression)
TRIBE: Cremolini 2015[93]	3	252 vs 256	First line	FOLFOXIRI plus bevacizumab vs FOLFIRI plus bevacizumab	65.0 vs 53.0 ($P = .006$; OR, 1.64)	12.1 vs 9.7 ($P = .003$; HR, 0.75)	31.0 vs 25.8 ($P = .054$; HR, 0.79)	Randomized phase 3 study in first-line treatment showing superiority of intensified triplet CT (FOLFOXIRI) plus bevacizumab compared with FOLFIRI plus bevacizumab in terms of ORR and PFS with a trend in favor of OS

(Continued)

TABLE 1. (Continued)

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
TRIBE 2: Cremolini 2017[96]	3	339 vs 340	First and second line	FOLFOXIRI plus bevacizumab and reintroduction vs FOLFOX plus bevacizumab followed by FOLFIRI plus bevacizumab	62.0 vs 50.0 ($P = .0023$; OR, 1.61)	mPFS1: 12.0 vs 9.8 ($P = .0002$; HR, 0.74); mPFS2: 19.2 vs 16.4 ($P = .0005$; HR, 0.74)	27.4 vs 22.5 ($P = .032$; HR, 0.82)	FOLFOXIRI plus bevacizumab as upfront strategy and the reintroduction of the same regimen is superior to a FOLFOX plus bevacizumab followed by FOLFIRI plus bevacizumab sequence strategy across 2 lines; primary end point, mPFS2
E3200: Giantonio 2007[97]	3	286 vs 291 vs 243	Second line	FOLFOX plus bevacizumab vs FOLFOX vs bevacizumab	22.7 vs 8.6 ($P < .0001$) vs 3.3	7.3 vs 4.7 ($P < .0001$; HR, 0.61) vs 2.7	12.9 vs 10.8 ($P < .0011$; HR, 0.75) vs 10.2	First randomized phase 3 study demonstrating that bevacizumab plus FOLFOX as second-line treatment significantly improves ORR, PFS, and OS in bevacizumab-naive patients
ML18147: Bennouna 2013[98]	3	409 vs 410	Second line	CT +/− bevacizumab after bevacizumab-based first line	5.0 vs 3.0 ($P = .573$)	5.7 vs 4.1 ($P < .0001$; HR, 0.68)	11.2 vs 9.8 ($P = .0062$; HR, 0.81)	The addition of bevacizumab to a second-line treatment also provided improved survival in patients who progressed to a bevacizumab-based first-line treatment; randomized phase 3 study demonstration of efficacy of bevacizumab *beyond progression* therapy.
VELOUR: Van Cutsem 2012[99]	3	612 vs 614	Second line	FOLFIRI plus ziv-aflibercept vs FOLFIRI	19.8 vs 11.1 ($P < .001$)	6.9 vs 4.7 ($P < .0001$; HR, 0.758)	13.5 vs 12.1 ($P = .0032$; HR, 0.817)	Ziv-aflibercept plus FOLFIRI significantly improves ORR, PFS, and OS as second-line therapy after FOLFOX +/− bevacizumab; higher gastrointestinal and antiangiogenic related toxicity compared with bevacizumab and ramucirumab.
RAISE: Tabernero 2015[100]	3	536 vs 536	Second line	FOLFIRI plus ramucirumab vs FOLFIRI	13.4 vs 12.5 ($P < .63$)	5.7 vs 4.5 ($P < .0005$; HR, 0.793)	13.3 vs 11.7 ($P = .0219$; HR, 0.844)	Ramucirumab plus FOLFIRI significantly improves PFS and OS as second-line therapy after FOLFOX plus bevacizumab.
Kemeny 2011[101]	2	38 vs 35	Adjuvant (after liver resection)	HAI plus FOLFIRI/FOLFOX +/− bevacizumab		1-y RFS, 71% vs 83%; 4-y RFS, 81% vs 85% ($P = .4$)	4-y OS, 81% vs 85% ($P = .5$)	Addition of bevacizumab to HAI plus systemic therapy after liver resection did not improve RFS and OS.

Abbreviations: +/−, with or without; 5-FU, 5-fluorouracil; CAPOX, capecitabine and oxaliplatin; CT, chemotherapy; exp, experimental; FOLFIRI, folinic acid, 5-fluorouracil, and irinotecan; FOLFOX, folinic acid, 5-fluorouracil, and oxaliplatin; HAI, hepatic arterial infusion; HR, hazard ratio; IFL, irinotecan, 5-fluorouracil bolus, and leucovorin; LV, leucovorin; mPFS, median progression-free survival; OR, odds ratio; ORR, overall response rate; OS, overall survival; PFS, progression free survival; RFS, recurrence-free survival; TTP, time to progression; WT, wild type.

TABLE 2. Key Phase 2 and 3 Trials With the Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Cetuximab in the First-Line or Second-Line Treatment of Unselected Patients With Metastatic Colorectal Cancer or According to the Presence or Absence of *RAS* Mutations

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	*RAS* MUTATION ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
CO.17: Jonker 2007,[106] Loree 2021[107]	Phase 3	572		Cetuximab/best supportive care vs best supportive care	Unselected patients	8.0 vs 0.0 ($P < .001$)	2.0 vs 2.0 ($P < .001$; HR, 0.68)	6.1 vs 4.6 ($P = .005$; HR, 0.77)	First randomized phase 3 study demonstrating that cetuximab monotherapy improves survival compared with best supportive care in unselected patients with chemorefractory mCRC.
					Extended *RAS/BRAF* WT	19.0 vs 0.0 ($P = .002$)	5.4 vs 1.8 ($P < .0001$; HR, 0.25)	10.1 vs 4.8 ($P = .004$; HR, 0.51)	Important post-hoc analysis that demonstrates a significant improvement in ORR, PFS, and OS only in extended *RAS/BRAF* WT mCRC.
CRYSTAL: Van Cutsem 2015[39]	Phase 3	1198	First line	FOLFIRI/cetuximab	Unselected patients	46.9 vs 38.7 ($P = .004$; OR, 1.40)	8.9 vs 8.0 ($P = .048$; HR, 0.85)	19.9 vs 18.6 ($P = .31$; HR, 0.93)	First phase 3 study to demonstrate that adding cetuximab to FOLFIRI as first-line treatment significantly improves ORR and PFS in patients with *KRAS* exon 2 WT mCRC. The benefit is increased in extended *RAS* analysis (KRAS exons 2, 3 and 4 and *NRAS* exons 2, 3 and 4), in which significantly improved OS is also demonstrated.
		367		FOLFIRI	Extended *RAS* WT	66.3 vs 38.6 ($P = .001$; OR, 3.11)	11.4 vs 8.4 ($P < .001$)	28.4 vs 20.2 ($P = .0024$; HR, 0.69)	
OPUS: Bokemeyer 2011[108]	Phase 2	337 vs 87	First line	FOLFOX cetuximab vs FOLFOX	Unselected patients	46.0 vs 36.0 ($P = .064$; OR, 1.516)	7.2 vs 7.2 ($P = .62$; HR, 0.931)	18.3 vs 18.0 ($P = .91$; HR, 1.015)	First phase 2 study to investigate the addition of cetuximab to FOLFOX as first-line treatment; no differences were shown in unselected patients with mCRC. In patients selected for extended *RAS* WT analysis, a significant difference in ORR, with a trend in PFS and OS, was observed with FOLFOX plus cetuximab.
					Extended *RAS* WT	58.0 vs 29.0 ($P = .0084$; OR, 3.33)	12.0 vs 5.8 ($P = .0615$; HR, 0.53)	19.8 vs 17.8 ($P = .8$; HR, 0.94)	
COIN: Maughan 2011[109]	Phase 3	751 of 2445	First line	5-FU/LV/oxaliplatin/cetuximab	*KRAS* exon 2 WT	64.0 vs 57.0 ($P = .049$; OR, 1.35)	8.6 vs 8.6 ($P = .60$; HR, 0.96)	17.0 vs 17.9 ($P = .67$; HR, 1.04)	Negative phase 3 trial for cetuximab plus capecitabine and oxaliplatin; potential influencing factor:, choice of backbone treatment (capecitabine is toxic in combination with cetuximab)
		581		5-FU/LV/oxaliplatin	Extended *RAS/BRAF* WT			19.9 vs 20.1 ($P = .86$; HR, 1.02)	

(Continued)

TABLE 2. (Continued)

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	*RAS* MUTATION ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
TAILOR: Qin 2018[110]	Phase 3	393	First line	FOLFOX/cetuximab vs FOLFOX	Extended *RAS* WT	61.1 vs 39.5 ($P < .001$; OR, 2.41)	9.2 vs 7.4 ($P = .004$; HR, 0.69)	20.7 vs 17.8 ($P = .02$; HR, 0.76	First randomized phase 3 study with cetuximab plus CT as first-line treatment in patients with mCRC who were prospectively selected for extended *RAS* WT tumor; significant improvement in ORR, PFS, and OS for the FOLFOX plus cetuximab arm
CALGB/SWOG 80405: Venook 2017[111]	Phase 3	429 (primary cohort)	First line	FOLFOX or FOLFIRI/cetuximab vs FOLFOX or FOLFIRI/bevacizumab	Extended *RAS* WT	68.8 vs 56.0 ($P < .01$)	11.2 vs 10.9 ($P = .43$; HR, 0.91)	31.8 vs 33.6 ($P = .38$; HR, 0.90)	No differences between cetuximab or bevacizumab plus first-line CT (FOLFOX, 75% of patients; FOLFIRI, 25% of patients). Post-hoc translational studies suggest that microsatellite status, tumor mutational burden, primary tumor localization (left vs right), *BRAF* V660E, and extended *RAS* mutations are factors that influence patient outcomes.
FIRE3: Heinemann 2014[112]	Phase 3	400	First line	FOLFIRI/cetuximab vs FOLFIRI/bevacizumab	Extended *RAS* WT	72.0 vs 56.1 ($P = .0029$; OR, 2.01)	8.7 vs 9.4 ($P = .53$; HR, 1.08)	33.1 vs 25.0 ($P = .0059$; HR, 0.70)	Randomized phase 3 study evaluating FOLFIRI in combination with either cetuximab or bevacizumab in unselected patients with mCRC. After extended *RAS* mutation stratification, patients with *RAS* WT tumor treated with cetuximab had significantly improved ORR and OS, but not PFS, compared with those treated with bevacizumab; first randomized study demonstrating that FOLFIRI plus cetuximab enhances early tumor shrinkage and depth of response, which correlate with improved OS
EPIC: Sobrero 2008[113]	Phase 3	648 vs 650	Second line after PD to oxaliplatin and 5-FU	Irinotecan/cetuximab vs irinotecan	Unselected	16.4 vs 4.2 ($P < .001$)	4.0 vs 2.6 ($P < .0001$)	10.7 vs 10.0 ($P = .975$)	Cetuximab-based second line determines better PFS and ORR; lack of benefit in OS could be related to the high number of patients in the irinotecan arm receiving cetuximab poststudy.

(Continued)

TABLE 2. (Continued)

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	*RAS* MUTATION ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
CAPRI: Ciardiello 2014[40] (first line)	Phase 2	182	First line	FOLFIRI/cetuximab	Extended *RAS* and *BRAF*, *PIK3CA* WT	64.4 (95% CI, 58.2-76.6)	11.3 (95% CI, 9.4-13.2)		Single-arm phase 2 study demonstrating antitumor activity of FOLFIRI plus cetuximab as first-line treatment in patients with *KRAS/NRAS/BRAF* and *PIK3CA* WT mCRC, as assessed by next-generation sequencing analysis of 22 genes
CAPRI: Ciardiello 2016[114] (second line)	Phase 2	153 vs 66	Second line after FOLFIRI/cetuximab	FOLFOX/cetuximab vs FOLFOX	Extended *RAS* and *BRAF*, *PIK3CA* WT	29.4 vs 9.4	6.9 vs 5.3 ($P = .025$; HR, 0.56)	23.7 vs 19.8 ($P = .056$; HR, 0.57)	Phase 2 study of cetuximab *beyond progression* in combination with FOLFOX as second-line treatment after progression to FOLFIRI plus cetuximab; significant improvement in ORR and PFS with a trend in OS for patients with *KRAS/NRAS/BRAF* and *PIK3CA* WT mCRC, as assessed by next-generation sequencing analysis of 22 genes
MACBETH: Cremolini 2019[115]	Phase 2	116	First line	FOLFOXIRI/cetuximab (plus cetuximab maintenance) vs FOLFOXIRI/cetuximab (plus bevacizumab maintenance)	Extended *RAS* and *BRAF* WT	71.6 (entire cohort)	10.1 vs 9.3 (HR, 0.83); 13.3 vs 10.8 (HR, 0.73)	33.2 vs 32.2 (HR, 0.92); 37.5 vs 37.0 (HR, 0.98)	Phase 2 study evaluating first-line therapy with CT triplet (FOLFOXIRI) plus cetuximab followed by cetuximab maintenance compared with the same regimen followed by bevacizumab maintenance in patients with mCRC selected for extended *RAS* and *BRAF* WT tumors

Abbreviations: 5-FU, 5-fluorouracil; CALGB, Cancer and Leukemia Group B; CI, confidence interval; CT, chemotherapy; FOLFOX, folinic acid, 5-fluorouracil, and oxaliplatin; FOLFOXIRI, folinic acid, 5-fluorouracil, oxaliplatin, and irinotecan; HR, hazard ratio; LV, leucovorin; mCRC, metastatic colorectal cancer; overall response rate; OR, odds ratio; OS, overall survival; PD, progressive disease; PFS, progression-free survival; SWOG, Southwest Oncology Group; WT, wild type.

TABLE 3. Key Phase 2 and 3 Trials With the Anti-Epidermal Growth Factor Receptor Monoclonal Antibody Panitumumab in the First-Line or Second-Line Treatment of Unselected Patients With Metastatic Colorectal Cancer or According to the Presence or Absence of RAS Mutations

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	*RAS* MUTATION ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
PRIME 098765: Douillard 2010[208], Douillard 2013[209]	Phase III	656 512	First-line	FOLFOX/panitumumab vs FOLFOX	*KRAS* exon 2 WT Extended *RAS* WT	55 vs 48 p= 0.068 OR: 1.35 Not reported for RAS WT	9.6 vs 8 p= 0.02 HR:0.80 10.1 vs 7.9 p= 0.004 HR:0.72	23.9 vs 19.7 p= 0.072 HR: 0.83 25.8 vs 20.2 p= 0.009 HR:0.77	First phase III trial showing significant increase in PFS by the addition of panitumumab to FOLFOX as first-line treatment in mCRC. In the extended *RAS* WT cohort (updated analysis) significant increase also in OS.
PEAK: Schwartzberg 2014 [210]	Phase II	285 170	First- line	FOLFOX/ panitumumab vs FOLFOX/bevacizumab	*KRAS* exon 2 WT Extended *RAS* WT	57.8 vs 53.5 63.6 vs 60.5	10.9 vs 10.1 p= 0.353 HR: 0.87 13.0 vs 9.5 p= 0.029 HR: 0.65	34.2 vs 24.3 p= 0.009 HR: 0.62 41.3 vs 28.9 p= 0.058 HR: 0.63	The first randomized phase II study to demonstrate better PFS and OS in patients with extended *RAS* WT tumors by the addition of panitumumab vs bevacizumab to FOLFOX as first-line therapy.
VOLFI: Modest 2019[211]	Phase II	96	First- line	FOLFOXIRI/ panitumumab vs FOLFOXIRI	Extended RAS WT	87.3.7 vs 60.6 p=0.004 HR: 4.469	9.7 vs 9.7 p= 0.76 HR: 1.07	35.7 vs 29.8 p= 0.12 HR:0.67	Adding panitumumab to an intensified CT regimen (FOLFOXIRI) confers a better ORR and radical resection of liver metastases, but PFS and OS were not improved.
VALENTINO: Pietrantonio 2019[212]	Phase II	229	First- line	FOLFOX/panitumumab (+ 5FU/ panitumumab maintenance) vs FOLFOX/panitumumab (+panitumumab maintenance)	Extended *RAS* WT	66.7 vs 67 p= 0.82 OR: 1.07	10 months PFS: 59.9 vs 49.0 p=0.009 HR: 1.51	18 months OS: 66.4% vs 62.4% p=0.60 HR: 1.13	Maintenance after first-line treatment with panitumumab alone is inferior to panitumumab plus 5-FU in terms of PFS following first-line therapy with FOLFOX + panitumumab.
PICCOLO: Seymour 2013[213]	Phase III	460 323	Second-line	Irinotecan/panitumumab vs panitumumab	*KRAS* exon 2 WT	34 vs 12 P<0.0001	p=0.015 HR= 0.78 HR:0.68	10.4 vs 10.5 p= 0.44 HR: 0.91 HR: 0.92	Primary endpoint (OS) not reached. Benefit in secondary endpoints (PFS and ORR) by the addition of panitumumab to irinotecan as second-line therapy in patients with *KAS* exon 2 tumors.
20050181: Peeters 2010[214], Peeters 2014[215]	Phase III	597 421	Second-line	FOLFIRI/panitumumab vs FOLFIRI	*KRAS* exon 2 WT Extended *RAS* WT	35 vs 10 P=0.001 41 vs 10	5.9 vs 3.9 p=0.004 HR: 0.73 6.4 vs 4.6 p=0.007 HR: 0.70	14.5 vs 12.5 P= 0.12 HR:0.85 16.2 vs 13.9 P=0.08 HR: 0.81	Randomized phase III study demonstrating improved ORR and PFS with a trend in OS by addition of panitumumab to FOLFIRI as second-line therapy for patients with extended *RAS* WT tumors. 41% ORR is the highest reported in phase III trials as second-line treatment.

(Continued)

TABLE 3. (Continued)

TRIAL	PHASE	NO. OF PATIENTS	SETTING	TREATMENT REGIMEN	*RAS* MUTATION ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
PLANET: Carrato 2017[216]	Phase II	77 53	First- line	FOLFOX/panitumumab vs FOLFIRI/panitumumab Liver limited disease	*KRAS* exon 2 WT Extended *RAS* WT	74 vs 67 p= 0.501 78 vs 73 p= 0.691	13 vs 14 p=0.728 HR: 0.90 13 vs 15 p= 0.307 HR: 0.7	37 vs 41 p =0.966 HR: 1.0 39 vs 49 p=0.824 HR:0.9	No difference of efficacy between FOLFOX-based or FOLFIRI-based combinations with panitumumab in patients with LLD.
PANAMA: Modest 2022[217]	Phase II	125 123	First-line	FOLFOX/panitumumab (+ 5-FU/ panitumumab maintenance) vs FOLFOX/panitumumab (+ 5-FU maintenance)	Extended *RAS* WT	40.8 vs 26 p= 0.02	8.8 vs 5.7 p= 0.014 HR: 0.72	28.7 vs 25.7 P= 0.32 HR: 0.84	Maintenance therapy with panitumumab + 5-FU improves ORR and PFS after first-line therapy with FOLFOX + panitumumab.
ASPECCT: Price 2014[218]	Phase III	499 500		Panitumumab versus cetuximab in patients with chemotherapy-refractory mCRC	*KRAS* exon 2 WT	22.0 vs 19.8	4.1 vs 4.1 HR: 1.00	10.4 vs 10p=0.0007 HR: 0.97	Randomized phase III study demonstrating that panitumumab is not inferior to cetuximab, providing similar ORR, PFS and OS in *KRAS* WT chemorefractory mCRC patients.

Abbreviations: 5-FU: 5-fluorouracil; ORR: overall response rate, mPFS: median progression free survival, mOS: median OS; HR: Hazard Ratio; OR: Odds Ratio; WT: Wild Type; CT: chemotherapy; LLD: liver limited disease.

generally consists of switching the chemotherapy backbone with the addition of an antiangiogenic drug. However, increasing numbers of clinical studies have suggested a possible role of anti-EGFR drug retreatment in the continuum of care of patients with *RAS/BRAF* WT mCRC. In fact, during second-line, EGFR-inhibitor–free treatment, acquired resistant *RAS*-mutant clones progressively decay (with a half-life of approximately 4 months), whereas *RAS/BRAF* WT clones may proliferate, restoring sensitivity to anti-EGFR drugs.[125] This has led to the concept of anti-EGFR therapy *rechallenge* (Table 4).[126-135] This potential third-line or fourth-line therapy may be offered to those patients who had a major response to first-line or second-line chemotherapy plus either cetuximab or panitumumab, after second-line or third-line treatment without EGFR inhibitors, respectively.[126] However, anti-EGFR therapy *rechallenge* should be considered only for those patients who have *RAS/BRAF* WT mCRC at the time of retreatment, as assessed by liquid biopsy analysis of plasma ctDNA.[127-130] Several clinical trials are currently ongoing to better define the activity and efficacy of anti-EGFR therapy *rechallenge*, with the aims of better identifying potentially responsive patients and of selecting the best treatment options (cetuximab or panitumumab as monotherapy or in combination with chemotherapy or with immune checkpoint inhibitors).[131-133,135]

Another relevant step toward a more personalized therapeutic approach has been the development of anti-BRAF–targeted therapies. Patients with *BRAF* V600E-mutant mCRC have the worst prognosis, with modest efficacy of chemotherapy regimens and with a median survival of only 12 months.[45,47,49] Several selective BRAF inhibitors have been developed and evaluated in clinical trials (Table 5).[51,136-142] A major therapeutic improvement was caused by the discovery that BRAF inhibition causes an escape mechanism in *BRAF*-mutant CRC cells, with the upregulation of EGFR-driven signaling, which allows cancer cell survival despite treatment.[142] Therefore, anti-BRAF monotherapy has limited therapeutic efficacy. In this respect, a pivotal, multicenter, randomized phase 3 study has demonstrated significant increases in objective responses, PFS, and OS after treatment with encorafenib, a selective BRAF inhibitor, in combination with the anti-EGFR monoclonal antibody cetuximab, in patients who have *BRAF* V600E mCRC after first-line or second-line therapy failure.[51] A still debated question is whether the efficacy of encorafenib plus cetuximab could be integrated with chemotherapy as first-line treatment.[143,144] This is being evaluated in a multicenter, randomized phase 3 study in which encorafenib plus cetuximab alone or in combination with FOLFOX is compared with FOLFOX/FOLFOXIRI plus bevacizumab as first-line therapy for patients with *BRAF* V600E mCRC.[145]

TABLE 4. Key Clinical Trials Evaluating Anti-Epidermal Growth Factor Receptor Rechallenge Therapy[a]

TRIAL	NO. OF PATIENTS	TREATMENT REGIMEN	*RAS* MUTATIONS ANALYSIS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	COMMENTS
Santini 2017[126] (retrospective)	39	Irinotecan or FOLFIRI plus cetuximab	*RAS* WT (archival tissue)	53.8	6.6	—	First retrospective study investigating rechallenge and/or anti-EGFR reintroduction in later lines for mCRC
CRICKET: Cremolini 2019[127] (prospective)	28	Irinotecan plus cetuximab	*RAS* WT (archival tissue)	21.4	3.49	9.8	First prospective, single-arm phase 2 study of irinotecan plus cetuximab rechallenge therapy as third-line treatment; liquid biopsy analysis with *RAS/BRAF* WT ctDNA before rechallenge could select responsive patients
	13		*RAS/BRAF* WT ctDNA	31.0	4.0	12.5	
	12		RAS-mutant ctDNA	0.0	1.9	5.2	
Sunakawa 2020[128] (prospective)	16	Irinotecan plus anti-EGFR	*RAS* WT (archival tissue)	0.0	3.1	8.9	Small size, single-arm study; liquid biopsy analysis with *RAS/BRAF* WT ctDNA before rechallenge could select responsive patients
	10		*RAS* WT ctDNA	0.0	4.7	16.0	
	6		*RAS*-mutant ctDNA	0.0	2.3	3.8	
CAVE: Martinelli 2021[129] (prospective)	77	Cetuximab plus avelumab	*RAS/BRAF* WT (archival tissue)	7.8	3.6	11.6	Largest prospective, single-arm phase 2 study; evaluation of cetuximab rechallenge plus the anti-PD-L1 monoclonal antibody avelumab in patients with MSS mCRC; of potential clinical relevance was the high OS in patients who had *RAS/BRAF* WT ctDNA assessed before rechallenge treatment.
	48		*RAS/BRAF* WT ctDNA	8.5	4.1	17.3	
	19		*RAS/BRAF* mutant ctDNA	5.1	3.0	10.4	
JACCRO-CC-08: Masuishi 2020[130] (prospective)	34	Irinotecan plus cetuximab	*KRAS* WT (archival tissue)	0.0	2.4	8.1	Single-arm, prospective study of cetuximab plus irinotecan rechallenge in third-line treatment; cetuximab-free interval could influence outcomes
Liu 2015[131] (retrospective)	89	Cetuximab +/− erlotinib	*KRAS* WT (archival tissue)	—	4.9 in prior responders, 2.9 in nonresponders	—	The study suggests that patients who had a prior response to cetuximab have a better outcome with rechallenge treatment
Tanioka 2018[132] (retrospective)	14	Irinotecan plus cetuximab	*KRAS* WT (archival tissue)	21.4	4.4	—	Retrospective analysis that confirmed better results with rechallenge strategy in patients who were good responders to first-line cetuximab
Rossini 2020[133] (retrospective)	86	Panitumumab; detuximab; FOLFIRI plus cetuximab; FOLFOX plus panitumumab; CapIRI plus cetuximab; irinotecan plus panitumumab; irinotecan plus cetuximab	RAS/BRAF WT (archival tissue)	19.8	3.8	10.2	Retrospective analysis of a multicenter, real-life database study
Karani 2020[134] (retrospective)	17	Cetuximab +/− chemotherapy	*KRAS* WT (archival tissue)	18.0	3.3	8.4	Study of re-induction and rechallenge with anti-EGFR–based treatment
Chong 2020[135] (retrospective)	22	Cetuximab or panitumumab	*KRAS* WT (archival tissue)	4.5	4.1	7.7	Retrospective analysis from the Australian mCRC Registry

Abbreviations: +/−, with or without; CapIRI, capecitabine and irinotecan; ctDNA: circulating tumor DNA; EGFR, epidermal growth factor receptor; FOLFIRI, folinic acid, 5-fluoroacil, and irinotecan; FOLFOX, folinic acid, 5-fluorouracil, and oxaliplatin; HR, hazard ratio; mCRC, metastatic colorectal cancer; MSS, microsatellite stability; ORR, overall response rate; OS, overall survival; PD-L1, programmed death ligand 1; WT, wild type.
[a]Rechallenge treatment is feasible in patients with *RAS* wild-type mCRC who have obtained a clinical benefit from previous anti-EGFR treatment, have progressed, and have received a subsequent *EGFR therapy-free* treatment.

TABLE 5. Key Clinical Trials of BRAF Inhibitors in Patients With *BRAF* V600E-Mutant Metastatic Colorectal Cancer

TRIAL	PHASE	NO. OF PATIENTS	TREATMENT REGIMEN	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
NCT00405587 (Kopetz 2015[136])	2	21	Vemurafenib	5.0	2.1	7.7	Targeting BRAF alone showed insufficient clinical activity in patients with *BRAF* V600E-mutated CRC.
NCT01791309 (Yager 2015[137])	2	15	Vemurafenib plus panitumumab	17.0	3.2	7.6	Considering that resistance to BRAF inhibitors was shown to be driven by feedback reactivation of EGFR in preclinical studies, this proof-of-concept exploratory study evaluated the efficacy and safety of panitumumab and vemurafenib in 15 chemorefractory patients with *BRAF* V600E-mutated CRC.
NCT01072175 (Corcoran 2015[138])	2	43	Dabrafenib plus trametinib	12.0	3.5	—	The study confirmed the synergistic activity of combining BRAF and MEK inhibition. However, the ORR was modest compared with their antitumor activity in melanoma.
NCT01750918 (Corcoran 2018[139])	2	20 vs 91 vs 31	Dabrafenib plus panitumumab (D+P) +/− trametinib (D+P+T)/(P+T):				Combined BRAF and EGFR and MEK inhibition was tolerable, with promising activity in patients with *BRAF* V600E-mutant mCRC. The trial confirmed that inhibition of key adaptive feedback pathways could prevent or overcome resistance to BRAF inhibition alone.
			D+P vs D+P+T vs P+T	10.0 vs 21.0 vs 0.0	3.5 vs 4.2 vs 2.6	13.2 vs 9.2 vs 9.1	
NCT01719380 (van Geel 2017[140])	2	26 vs 28	Encorafenib plus cetuximab +/− alpelisib	19.2 vs 17.9	3.7 vs 4.2	—	The study evaluated whether PI3K inhibition could add any benefit to BRAF and EGFR inhibition. Dual-combination and triple-combination treatments showed similar clinical efficacy, despite increased toxicity with the triple combination.
SWOG 1406 (Kopetz 2017[141])	2	106	Vemurafenib plus cetuximab plus irinotecan vs cetuximab plus irinotecan	17.0 vs 4.0 ($P = .05$)	4.4 vs 2.0 ($P < .001$; HR, 0.50)		First randomized phase 2 study in chemorefractory patients with *BRAF* V600E mCRC demonstrating significant increases in ORR and PFS with the addition of the BRAF inhibitor vemurafenib to irinotecan plus cetuximab therapy
BEACON (Kopetz 2019[51])	3	224 (triplet); 220 (doublet); 221 (control)	Encorafenib plus cetuximab +/− binimetinib (triplet and doublet) vs irinotecan plus cetuximab (control)	26.8 and 19.5 vs 1.8	4.5 (HR, 0.42 vs control) and 4.3 (HR, 0.44 vs control) vs 1.5	9.3 (HR, 0.60 vs control) and 9.3 (HR, 0.61 vs control) vs 5.9	The first randomized phase 3 trial to demonstrate that a combination of molecular target therapies (cetuximab plus the BRAF inhibitor encorafenib +/− the MEK inhibitor binimetinib) resulted in a significant and clinically relevant benefit compared with current standard therapy in patients with *BRAF* V600E-mutant mCRC who had had disease progression after 1 or 2 previous regimens, by increasing ORR, PFS, and OS. Triplet and doublet combinations produced similar patient outcomes; therefore, both the FDA and the EMA have approved the doublet combination with encorafenib plus cetuximab.
ANCHOR (Prahallad 2012[142])	2	90	First line: encorafenib plus cetuximab plus binimetinib	47.8 (95% CI, 37.3-58.5)	5.8 (95% CI, 4.6-6.4)	17.2 (95% CI, 14.1-21.1)	The first single-arm, phase 2 study to evaluate BRAF inhibitor-based therapy as first-line treatment for patients with *BRAF* V600E mCRC; encorafenib plus cetuximab plus binimetinib treatment results in potentially clinical relevant ORR, PFS, and OS.

Abbreviations:+/ −, with or without; CI, confidence interval; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; FDA, US Food and Drug Administration; HR, hazard ratio; mCRC, metastatic colorectal cancer; MEK, MAP/ERK kinase; NCT, ClinicalTrials.gov identifier; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PI3K, phosphoinositide-3 kinase; SWOG, Southwest Oncology Group.

TABLE 6. Key Clinical Trials Exploring the Efficacy of Anti-HER2 Treatments in Metastatic Colorectal Cancer

TRIAL	PHASE	NO. OF PATIENTS	TREATMENT REGIMEN	HER2 AMPLIFICATION OR OVEREXPRESSION	*RAS* STATUS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
HERACLES A (Sartore-Bianchi 2016[146])	2	27	Trastuzumab plus lapatinib	IHC3+ in 50% of cells or IHC2+/FISH-positive (HER2:CEP17 ratio, >2 in >50% of cells)	*KRAS* WT	28.0	5.4 (4.7 for ERBB2 CNG >9.5; 3.7 for ERBB2 CNG <9.5)	10.0	The first single-arm phase 2 study to demonstrate potentially clinical relevant antitumor activity of dual HER2 blockade with trastuzumab plus lapatinib in chemorefractory patients with *HER2*-amplified mCRC.
HERACLES B (Sortore-Bianchi 2020[147])	2	31	T-DM1 plus pertuzumab	IHC3+ in 50% of cells or IHC2+/FISH-positive (HER2:CEP17 ratio, >2 in >50% of cells)	Extended *RAS/BRAF* WT	10.0	4.8	—	The combination of pertuzumab and T-DM1 did not reach the ORR primary end point. However, based on good disease control, PFS, and low toxicity, it can be considered as an alternative therapeutic approach in chemorefractory patients with *HER2*-amplified mCRC.
MyPathway (Meric-Bernstam 2019[148])	2	56; 40	Trastuzumab plus pertuzumab	IHC3+/FISH-positive (HER2:CEP17 ratio > 2)	Unselected	32.0	2.9	11.5	Results of MyPathway basket trial demonstrated that dual *HER2*-targeted therapy of pertuzumab plus trastuzumab was effective in a wide variety of *HER2* amplified or overexpressing tumors, including chemorefractory mCRC.
		40		HER2 amplification and/or NGS CNG	*KRAS* WT	40.0	5.3	14.0	
MOUNTAINEER (Strickler 2019[149])	2	26	Trastuzumab plus tucatinib	IHC3+ or IHC2+/FISH-positive or NGS	Extended *RAS* WT	55.0	6.2	17.3	MOUNTAINEER is an ongoing phase 2-phase 3 program evaluating the combination of trastuzumab with the anti-HER2 tyrosine kinase inhibitor tucatininb. Promising preliminary results in terms of ORR, PFS, and OS have been presented.
TRIUMPH (Nakamura 2019[150])	2	19	Trastuzumab plus pertuzumab	NGS selection on tissue and ctDNA	Extended *RAS* WT	Tissue, 35.0; ctDNA, 33.0	4.0	—	First single-arm, small size, proof-of-concept phase 2 trial to prospectively evaluate the efficacy of dual monoclonal antibody *HER2*-blockade (trastuzumab plus pertuzumab) in chemorefractory mCRC with *HER2* amplification, as assessed by either tissue or ctDNA analysis, suggesting a role of liquid biopsy in selecting patients for treatment.
DESTINY-CRC01 (Siena 2021[151])	2	53	Trastuzumab-deruxtecan	IHC/FISH: cohort A, IHC3+/IHC2+, FISH-positive	Extended *RAS/BRAF* V600E WT	45.3 (95% CI, 31.6-59.6)	6.9 (95% CI, 4.1-8.7)	15.5 (95% CI, 8.8-20.8)	First prospective single-arm, phase 2 study of trastuzumab-deruxtecan in patients who had chemorefractory mCRC with *HER2* amplification and/or overexpression; potentially clinical relevant activity in terms of ORR, PFS, and OS

Abbreviations: CI, confidence interval; CNG, copy number gain; ctDNA, circulating tumor DNA; FISH, fluorescent in situ hybridization; IHC3+ immunohistochemistry score ≥ 3; NGS, next-generation sequencing; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; T-DM1, trastuzumab emtansine; WT, wild type.

TABLE 7. Initial Early Phase Clinical Studies With *KRAS* G12C Inhibitors (Sotorasib and Adagrasib) in Monotherapy or in Combination With Anti-Epidermal Growth Factor Receptor Drugs in Metastatic Colorectal Cancer

TRIAL	PHASE	NO. OF PATIENTS	TREATMENT REGIMEN	DCR, %	ORR, %	MEDIAN PFS, MONTHS	HIGHLIGHTS
CodeBreaK100 (Hong 2020[154])	1	129, of which 42 had *KRAS* G12C mCRC	Sotorasib	78.3	7.1	4.0	Sotorasib was the first selective *KRAS* G12C inhibitor with potentially relevant anticancer activity in patients with chemorefractory mCRC.
		Dose-exploration cohort	Sotorasib plus panitumumab +/− FOLFIRI (ongoing)				
CodeBreaK100 (Fakih 2022[152])	2	62	Sotorasib		9.7	4.8	The first single-arm phase 2 study extended the information on sotorasib monotherapy in terms of antitumor activity and safety profile in patients with chemorefractory mCRC and a *KRAS* G12C mutation.
KRYSTAL-1 (Weiss 2021[155])	2	45	Adagrasib monotherapy (phase 1/2)		87.0	22.0	The multicohort phase 1/2 KRYSTAL-1 study showed encouraging clinical activity with adagrasib as monotherapy as well as increased clinical activity in combination with cetuximab in patients who had chemorefractory mCRC with *KRAS* G12C mutation.
		28	Adagrasib plus cetuximab (first data cutoff analysis)		100.0	43.0	

Abbreviations: +/−, with or without; DCR, disease-control rate; FOLFIRI, folinic acid, 5-fluoroacil, and irinotecan; mCRC, metastatic colorectal cancer.

The identification of *HER2* gene amplification in a small subgroup of patients with *RAS/BRAF* WT mCRC has led to a series of clinical trials that have tested different anti-HER2 therapeutic approaches (Table 6).[146-151] On the basis of elegant preclinical studies with patient-derived tumor xenografts, the combination of trastuzumab, a humanized anti-HER2 monoclonal antibody, with lapatinib, an anti-HER2 tyrosine kinase inhibitor, has been the first successful, effective therapy in chemorefractory patients with *HER2*-amplified mCRC.[62] Other promising approaches include the combination of trastuzumab with the humanized anti-HER2 monoclonal antibody pertuzumab and, more recently, the use of trastuzumab-deruxtecan, in which the topoisomerase I inhibitor deruxtecan is covalently linked to trastuzumab.[147,151] Finally, antitumor activity also has been observed with the combination of trastuzumab plus the selective anti-HER2 tyrosine kinase inhibitor tucatinib.[149] Because the efficacy of anti-HER2 therapies has been established in the treatment of chemorefractory patients, clinical trials are currently ongoing to evaluate their potential role in earlier phases, including first-line therapy.

KRAS mutations are the most frequent oncogenic mutations in mCRC, occurring in >40% of cases.[3] Until recently, *KRAS* has been considered an *undruggable* oncogenic driver. All of the efforts to develop selective KRAS inhibitors were unsuccessful for long time. The first generation of potential KRAS inhibitors was represented by farnesyltransferase inhibitors, which were developed to block the post-translational modifications of KRAS that are needed for protein localization to the inner face of the cell membrane, which is crucial for its activity.[43] However, these drugs failed in clinical development. A different class of KRAS inhibitors has been developed since 2013, rapidly opening a new therapeutic scenario. These are selective, irreversible inhibitors of the *KRAS* G12C mutant, which occurs in approximately 3% to 4% mCRCs.[44] Recently, 2 drugs, sotorasib and adagrasib, have been approved by the FDA for the treatment of *KRAS* G12C-mutant, advanced lung cancer and are currently under extensive clinical investigation in mCRC (Table 7).[152-155] Although signs of clinical activity with either sotorasib or adagrasib monotherapy have been reported in chemorefractory patients with *KRAS* G12C mCRC, preclinical studies strongly support the need for combining these inhibitors with anti-EGFR monoclonal antibodies to overcome cancer cell escape mechanisms caused by the activation of EGFR signaling when they are used as single agents. In this respect, signals of better antitumor activity of these combinations in early phase clinical studies have generated 2 large, randomized phase 3 trials, which are currently ongoing, to evaluate the efficacy of sotorasib plus panitumumab as third-line therapy or adagrasib plus cetuximab as second-line therapy in patients who have *KRAS* G12C mCRC compared with

TABLE 8. Key Clinical Trials With Immune Checkpoint Inhibitors in Patients With Microsatellite Instability-High/DNA Mismatch Repair-Deficient or Microsatellite Stability/DNA Mismatch Repair-Proficient Metastatic Colorectal Cancer

TRIAL	PHASE	NO. OF PATIENTS	TREATMENT REGIMEN	MICROSATELLITE STATUS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
KEYNOTE 016 (Le 2015[166])	2	41 (32 with mCRC)	Pembrolizumab in chemorefractory patients	MSI-H/dMMR; MSS	40.0	NR	NR	The first clinical evidence of pembrolizumab activity and efficacy in MSI-H/dMMR tumors compared with MSS/pMMR tumors, proposing MSI-H/dMMR as a biomarker for immunotherapy
				MSS	0.0	2.2	5.0	
KEYNOTE 164 (Toyota 1999[21])	2	63	Pembrolizumab in chemorefractory patients	MSI-H/dMMR	32.0	4.1	76.0% at 12 mo	This study confirmed the durable clinical benefit of pembrolizumab in patients with previously treated MSI-H/dMMR mCRC.
KEYNOTE 177 (Andre 2020[167])	3	307	Pembrolizumab vs CT as first-line therapy	MSI-H/dMMR	43.8 vs 33.1	16.5 vs 8.2 ($P = .0002$; HR, 0.60)	Median survival NR: 61% of patients alive at 36 mo with pembrolizumab	The first randomized, prospective phase 3 study of first-line therapy in MSI-H/dMMR mCRC. Pembrolizumab determines highly clinically relevant and statistically significant improvement in ORR, PFS, and OS (major trend in favor) compared with standard CT +/− target therapies: A new standard of care of precision medicine-based immunotherapy.
NCT02375672 (Shitara 2020[168])	2	30	Pembrolizumab plus FOLFOX as first line	mCRC (3% with MSI-H/dMMR)	56.7	8.8	NR	Pembrolizumab can be safely combined with CT in patients with mCRC.
CheckMate 142 (Overman 2017[164])	2	74	Nivolumab (chemorefractory patients)	MSI-H/dMMR	31.1	50.0% (12-mo PFS rate)	73.0% (12-mo OS rate)	Nivolumab plus low-dose ipilimumab demonstrated improved clinical benefit compared with nivolumab monotherapy, with a favorable benefit-risk profile, in chemorefractory patients with MSI-H/dMMR mCRC.
		119	Nivolumab plus ipilimumab (chemorefractory patients)		55.0	71.0% (12-mo PFS rate)	85.0% (12-mo OS rate)	
CheckMate 142 (Lenz 2022[169])	2	45	Nivolumab plus ipilimumab as first line	MSI-H/dMMR	69.0 (95% CI, 53.0-82.0)	74.0% at 24 mo	79.0% at 24 mo	Nivolumab plus low-dose ipilimumab was effective as first-line therapy (single-arm, phase 2 data).
IMblaze 370 (Eng 2019[170])	3	90 vs 183 vs 90	Atezolizumab vs cobimetinib/atezolizumab vs regorafenib in chemorefractory patients	MSS (MSI-H in <5% of patients)	2.0 vs 3.0 vs 2.0	1.94 vs 1.91 vs 2.0	7.1 vs 8.87 vs 8.5	Randomized phase 3 IMblaze370 trial failed to meet its primary end point, showing no increase in survival for patients treated with an anti–PD-L1 monoclonal antibody (atezolizumab) in combination with an MEK inhibitor (cobimetinib) compared with standard of care (regorafenib).

(Continued)

TABLE 8. (Continued)

TRIAL	PHASE	NO. OF PATIENTS	TREATMENT REGIMEN	MICROSATELLITE STATUS	ORR, %	MEDIAN PFS, MONTHS	MEDIAN OS, MONTHS	HIGHLIGHTS
CCTG CO.26 (Chen 2020[171])	2	119 vs 161	Durvalumab/tremelimumab vs BSC in chemorefractory patients	MSS	0.5 vs 0.0	1.8 vs 1.9	6.6 vs 4.1 ($P = .07$)	Combination treatment with anti–PD-L1 (durvalumab) and anti–CTLA-4 (tremelimumab) monoclonal antibodies in unselected, chemorefractory mCRC with a trend in better OS compared with BSC
REGONIVO (Fukuoka 2020[172])	1b	36	Regorafenib plus nivolumab in chemorefractory patients	MSS	36.0	7.9	—	Clinical signals of activity of regorafenib plus nivolumab combination in patients with chemorefractory MSI-H/pMMR mCRC; it is an early phase (1b) study with a limited number of patients; results have not been confirmed in a larger, single-arm phase 2 study.
ATEZOTRIBE (Cremolini 2021[173])	2	132 vs 67	FOLFOXIRI/bevacizumab plus atezolizumab vs FOLFOXIRI/bevacizumab as first line	MSS and MSI-H/dMMR	59.0 vs 64.0 ($P = .412$)	13.1 vs 11.5 ($P = .012$; HR, 0.69)	NR	Randomized phase 2 study of adding an anti–PD-L1 monoclonal antibody (atezolizumab) to intensified CT (FOLFOXIRI) plus bevacizumab as first-line therapy for the treatment of patients with mCRC; PFS advantage in the experimental arm; survival data not yet mature
MAYA (Pietrantonio 2021[174])	2	33	Temozolomide plus nivolumab plus ipilimumab in refractory patients	MSS	42.0	7.1	18.4	Strategy for immune-sensitizing chemorefractory mCRC with the cytotoxic drug temozolomide in patients with MSS/MGMT-silenced mCRC; promising signals of activity and efficacy

Abbreviations: +/−, with or without; BSC, best supportive care; CCTG, Canadian Cancer Trials Group; CT, chemotherapy; dMMR, DNA mismatch-repair–deficient; FOLFOX, folinic acid, 5-fluorouracil, and oxaliplatin; FOLFOXIRI, folinic acid, 5-fluorouracil, oxaliplatin, and irinotecan; HR, hazard ratio; mCRC, metastatic colorectal cancer; MEK, MAP/ERK kinase; MGMT, O-6-methylguanine-DNA methyltransferase; mOS, median overall survival; mPFS, median progression-free survival; MSI, microsatellite instability; MSI-H, high microsatellite instability; MSS, microsatellite stability; NR, not reached; ORR, overall response rate; OS, overall survival; PD-L1, programmed death ligand 1; PFS, progression-free survival; pMMR, DNA mismatch-repair–proficient.

appropriate standard therapies. This is only the beginning of a new therapeutic era. However, targeting the most frequent activating *RAS* mutations remains a major unmet medical need in mCRC.

NTRK fusions are very rare gene alterations in mCRC.[66,156] However, the development of 2 inhibitors (entrectinib and larotrectinib) that received recent approval by the FDA and the European Medicines Agency for their therapeutic use, which is based on the presence of the specific gene alteration regardless of tumor type, in a so-called *agnostic* indication, offers another relevant treatment opportunity for chemorefractory patients who have mCRC with *NTRK* fusion.[157,158]

In this respect, for patients with chemorefractory mCRC, the use of molecularly selected treatments based on the presence of *druggable* tumor gene alterations (as in the case of anti-EGFR *rechallenge*) for anti-*BRAF* V600E, anti-HER2, anti-*KRAS* G12C, or anti-*NTRK* therapies is a clinically relevant goal. In fact, the current treatment options after progression on second-line therapy rely on the use of either regorafenib or trifluridine-tipiracil, a fluoropyrimidine-derivative drug, which have been established as third-line or fourth-line therapies for unselected patients with mCRC.[102,159,160] Randomized phase 3 trials have shown that treatment with either regorafenib or trifluridine-tipiracil determines a statistically significant, but clinically modest, increase in survival compared with best supportive care.[102,159] Therefore, the application of a precision medicine-based approach could allow an opportunity to provide further effective line(s) of selected therapies before treatment with regorafenib and/or trifluridine-tipiracil.

Integrating Immunotherapies in the Continuum of Care of Metastatic Colorectal Cancer

Evaluation of microsatellite status or of the mismatch-repair machinery is currently mandatory before treatment decision choices are made for mCRC first-line therapy.[161] MSI-H/dMMR mCRC tumors are characterized by high numbers of neoantigens, which increase immunogenicity, are associated with a high immune infiltrate in the tumor microenvironment, and, in turn, may render these tumors amenable to immune checkpoint inhibitor therapy.[162] MSI-H/dMMR is the first predictive biomarker of immunotherapy efficacy for mCRC. Although these tumors are a small subgroup of mCRC (approximately 5% of cases), the introduction of immune checkpoint inhibitors has been a revolution in the precision medicine approach to mCRC therapies.[163] Two anti–PD-1 monoclonal antibodies—pembrolizumab and nivolumab—and the combination of nivolumab plus the anti–CTLA-4 monoclonal antibody ipilimumab have been approved by the FDA for the treatment of patients with MSI-H/dMMR mCRC.[164] According to phase 2 data,[165] the combination of nivolumab plus ipilimumab seems to be more effective in terms of increased objective responses, disease control, and survival compared with nivolumab monotherapy (Table 8).[21,164,166-174] The results of a multicenter, randomized phase 3 trial, in which pembrolizumab was compared with standard therapy as first-line treatment, have demonstrated a highly statistically significant doubling in median PFS, which reached 16.5 months, in pembrolizumab-treated patients.[167] This is the longest median PFS reported to date in randomized phase 3 trials for any first-line treatment in mCRC. Furthermore, the majority of pembrolizumab-treated patients achieved major objective responses of long duration (84% lasted ≥2 years).

According to the CMS classification, MSI-H/dMMR mCRC belongs to the *immune-activated* CMS1 subgroup. Conversely, immunotherapy is not effective in microsatellite stable (MSS)/pMMR tumors, which are approximately 95% of mCRCs. MSS/pMMR tumors display a low number of neoantigens, with generally low immune infiltrate in the microenvironment, poor expression of MHC molecules, and, consequently, lack of sensitivity to immune checkpoint inhibitor blockade.[41] These mCRCs could be classified in the CMS2 and CMS3 subgroups, which are defined as an *immune desert*, or in the CMS4 subgroup, which is characterized by an *inflamed* tumor microenvironment with enhanced TGF-β signaling, increased angiogenesis, and EMT.[78]

Therefore, a major challenge for the clinical management of patients with mCRC is to find novel therapeutic approaches that could render immune-competent those tumors that are designated as an *immune desert* or as *inflamed*. It has been shown that cytotoxic drugs, antiangiogenic agents, molecular target therapies, and radiotherapy could activate immunogenic cell death (ICD) in cancer cells. ICD could determine the release of a high numbers of tumor antigens as well as proinflammatory cytokines, which increase the immune infiltrate in the tumor microenvironment.[175-177] Moreover, ICD may cause the activation and maturation of antigen-presenting cells, including macrophages and dendritic cells, with the final result of eliciting specific immune responses against tumor antigens.[178,179] Taken together, these observations support the hypothesis that a combination of immune checkpoint inhibitors and anticancer therapies could overcome primary resistance of MSS/pMMR mCRC to immunotherapy. Several clinical studies are investigating the potential role of immune checkpoint inhibitors in combination with chemotherapy, including combinations with antiangiogenic drugs (monoclonal antibodies and multikinase inhibitors); anti-EGFR monoclonal antibodies; inhibitors of intracellular signal-transduction pathways, including *KRAS* G12C, *BRAF* V600E, or MEK inhibitors; or radiotherapy.[129,171-174,180-190] Although promising signs of therapeutic activity have been observed in some of these

studies, further translational and clinical investigation is needed to identify the most effective combination strategies as well to appropriately select the patients who could obtain a clinical benefit from this approach.

Next Steps for Implementing Precision Medicine in the Clinical Management of Metastatic Colorectal Cancer

The molecular stratification on which the current treatment algorithm for mCRC is based does not fully represent the complex and heterogeneous genotypes and phenotypes of this disease. A more informative profiling would be needed to implement more effective and individualized therapies for each patient. Therefore, the next steps in the clinical management of patients with mCRC will be to integrate the comprehensive knowledge of tumor gene alterations, tumor and microenvironment gene and protein expression, host immune competence, and their dynamic changes throughout the disease course at an individual patient level for a truly precision medicine-based continuum of care.

The first model of human CRC development and progression, which was described by Fearon and Vogelstein in 1990, was based on a progressive, linear sequence of genetic events involving oncogenes and tumor-suppressor genes.[13] We are now moving to a multiparametric approach for better explaining the disease network complexity through the study of transcriptomics, proteomics, metagenomics, and radiomics.[191]

The first attempt has been the development of a molecular classification that includes not only genetic alterations but also transcriptomics features, such as gene expression profiles, microRNA patterns, and methylation status, of both cancer cells and the tumor microenvironment. These efforts have resulted in the CMS classification.[72] Subsequent studies have been focused on proteomics, with the aim of identifying protein panels involved in mCRC dynamics.[192-194] Unfortunately, we are still far from the discovery of prognostic and predictive proteomic biomarkers for mCRC that could be validated in the clinical setting. This is in part because of the complex interpretation of results, which are often heterogeneous or even discordant among different studies.

Metagenomics studies have highlighted the role of the gut microbial community in CRC pathogenesis as well as in modulating response to anticancer therapies.[195] In fact, another factor of complexity that should be taken in account for a more comprehensive definition of mCRC in the era of precision medicine is gut microbiota, the presence of which has recently been proposed as a key factor in the dynamic process of acquisition of the hallmarks of cancer.[196] Trillions of microbes, including bacteria, are physiologically hosted in the human gastrointestinal tract. Gram-negative *Bacteroidetes* and Gram-positive *Firmicutes* constitute approximately 90% of gut microbiota in healthy conditions. In a physiologic balance with the host, gut microbiota produce useful metabolites, including vitamins and short-chain fatty acids, such as butyrate, to support the integrity of the intestinal epithelial barrier and of host immune defenses.[197] Preclinical and clinical research has highlighted an emerging role for intestinal bacteria species in CRC development and progression as well as in response to anticancer treatments.[198] In this respect, gut microbiota have been implicated in cancer immunotherapy activity and toxicity.[199-201] The short-chain fatty acid butyrate, which can be produced by selected intestinal bacteria species, is a potential modulator of host innate and adaptive immunity in both physiologic and pathologic conditions.[202,203] A major clinical challenge is to develop suitable therapeutic approaches with the aim of increasing immune checkpoint inhibitor activity and efficacy through the modulation of gut microbiota in patients with mCRC.

Finally, an emerging field of research comes with the implementation of radiomic analyses of mCRC. Radiogenomic models, which are developed from diagnostic imaging data, could identify radiomic signatures of antitumor activity after treatment with molecular target drugs or immunotherapy.[204-207]

In this scenario, the future will be the development of a global functional and dynamic *multiomic* classification of mCRC. This will require major efforts for implementing standardized and reproducible technologies before it can be applied to clinical practice. However, this classification approach could result in individually defined prognostic and predictive parameters, which could help the clinician in choosing the most appropriate therapeutic program(s) throughout the entire disease journey for each patient with mCRC. ■

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin.* 2022;72:7-33. doi:10.3322/caac.21708
2. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. *CA Cancer J Clin.* 2020;70:145-164. doi:10.3322/caac.21601
3. Biller LH, Schrag D. Diagnosis and treatment of metastatic colorectal cancer: a review. *JAMA.* 2021;325:669-685. doi:10.1001/jama.2021.0106
4. Global Burden of Disease 2019 Cancer Collaboration; Kocarnik JM, Compton K, Dean FE, et al. Cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life years for 29 cancer groups from 2010 to 2019: a systematic analysis for the Global Burden of Disease Study 2019. *JAMA Oncol.* 2021;8420-444. doi:10.1001/jamaoncol.2021.6987
5. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. *Lancet.* 2019;394:1467-1480. doi:10.1016/S0140-6736(19)32319-0

6. Riedesser JE, Ebert MP, Betge J. *Precision medicine for metastatic colorectal cancer in clinical practice. Ther Adv Med Oncol. Published online January* 19, 2022. doi:10.1177/17588359211072703
7. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. *N Engl J Med*. 1988;319:525-532. doi:10.1056/NEJM198809013190901
8. Buikhuisen JY, Torang A, Medema JP. Exploring and modelling colon cancer inter-tumour heterogeneity: opportunities and challenges. *Oncogenesis*. 2020;9:66. doi:10.1038/s41389-020-00250-6
9. Di Nicolantonio F, Vitiello PP, Marsoni S, et al. Precision oncology in metastatic colorectal cancer—from biology to medicine. *Nat Rev Clin Oncol*. 2021;18:506-525. doi:10.1038/s41571-021-00495-z
10. Punt CJ, Koopman M, Vermeulen L. From tumour heterogeneity to advances in precision treatment of colorectal cancer. *Nat Rev Clin Oncol*. 2017;14:235-246. doi:10.1038/nrclinonc.2016.171
11. Pino MS, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology*. 2010;138:2059-2072. doi:10.1053/j.gastro.2009.12.065
12. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature*. 1998;396:643-649. doi:10.1038/25292
13. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*. 1990;61:759-767. doi:10.1016/0092-8674(90)90186-i
14. Samuels Y, Wang Z, Bardelli A, et al. High frequency of mutations of the PIK3CA gene in human cancers. *Science*. 2004;304:554. doi:10.1126/science.1096502
15. Gerstung M, Jolly C, Leshchiner I, et al. The evolutionary history of 2,658 cancers. *Nature*. 2020;578:122-128. doi:10.1038/s41586-019-1907-7
16. Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol*. 2006;7:335-346. doi:10.1038/nrm1907
17. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology*. 2010;138:2073-2087. e3. doi:10.1053/j.gastro.2009.12.064
18. Malki A, ElRuz RA, Gupta I, Allouch A, Vranic S, Al Moustafa AE. Molecular mechanisms of colon cancer progression and metastasis: recent insights and advancements. *Int J Mol Sci*. 2020;22:130. doi:10.3390/ijms22010130
19. Bonadona V, Bonaiti B, Olschwang S, et al. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. *JAMA*. 2011;305:2304-2310. doi:10.1001/jama.2011.743
20. Ijspeert JEG, Vermeulen L, Meijer GA, Dekker E. Serrated neoplasia-role in colorectal carcinogenesis and clinical implications. *Nat Rev Gastroenterol Hepatol*. 2015;12:401-409. doi:10.1038/nrgastro.2015.73
21. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. *Proc Natl Acad Sci U S A*. 1999;96:8681-8686. doi:10.1073/pnas.96.15.8681
22. Weisenberger DJ, Siegmund KD, Campan M, et al. CpG island methylator phenotype underlies sporadic microsatellite instability and is tightly associated with BRAF mutation in colorectal cancer. *Nat Genet*. 2006;38:787-793. doi:10.1038/ng1834
23. Suzuki H, Igarashi S, Nojima M, et al. IGFBP7 is a p53-responsive gene specifically silenced in colorectal cancer with CpG island methylator phenotype. *Carcinogenesis*. 2010;31:342-349. doi:10.1093/carcin/bgp179
24. Kriegl L, Neumann J, Vieth M, et al. Up and downregulation of p16(Ink4a) expression in BRAF-mutated polyps/adenomas indicates a senescence barrier in the serrated route to colon cancer. *Mod Pathol*. 2011;24:1015-1022. doi:10.1038/modpathol.2011.4
25. Ogino S, Kawasaki T, Kirkner GJ, Suemoto Y, Meyerhardt JA, Fuchs CS. Molecular correlates with MGMT promoter methylation and silencing support CpG island methylator phenotype-low (CIMP-low) in colorectal cancer. *Gut*. 2007;56:1564-1571. doi:10.1136/gut.2007.119750
26. Yaeger R, Chatila WK, Lipsyc MD, et al. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell*. 2018;33:125-136.e3. doi:10.1016/j.ccell.2017.12.004
27. Demetri GD, von Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med*. 2002;347:472-480. doi:10.1056/NEJMoa020461
28. Herbst R, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature*. 2018;553:446-454. doi:10.1038/nature2518
29. Holderfield M, Deuker M, McCormick F, et al. Targeting RAF kinases for cancer therapy: BRAF-mutated melanoma and beyond. *Nat Rev Cancer*. 2014;14:455-467. doi:10.1038/nrc3760
30. Troiani T, Napolitano S, Della Corte CM, et al. Therapeutic value of EGFR inhibition in CRC and NSCLC: 15 years of clinical evidence. *ESMO Open*. 2016;1:e000088. doi:10.1136/esmoopen-2016-000088
31. Ciardiello F, Tortora G. EGFR antagonists in cancer treatment [erratum in: *N Engl J Med*. 2009;360:1579]. *N Engl J Med*. 2008;358:1160-1174. doi:10.1056/NEJMra0707704
32. Normanno N, Tejpar S, Morgillo F, De Luca A, Van Cutsem E, Ciardiello F. Implications for KRAS status and EGFR-targeted therapies in metastatic CRC. *Nat Rev Clin Oncol*. 2009;6:519-527. doi:10.1038/nrclinonc.2009.111
33. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. *Cell*. 2017;170:17-33. doi:10.1016/j.cell.2017.06.009
34. Dias Carvalho P, Guimaraes CF, Cardoso AP, et al. KRAS oncogenic signaling extends beyond cancer cells to orchestrate the microenvironment. *Cancer Res*. 2018;78:7-14. doi:10.1158/0008-5472.CAN-17-2084
35. Lievre A, Bachet JB, Le Corre D, et al. KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. *Cancer Res*. 2006;66:3992-3995. doi:10.1158/0008-5472.CAN-06-0191
36. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. *J Clin Oncol*. 2008;26:1626-1634. doi:10.1200/JCO.2007.14.7116
37. Benvenuti S, Sartore-Bianchi A, Di Nicolantonio F, et al. Oncogenic activation of the RAS/RAF signaling pathway impairs the response of metastatic colorectal cancers to anti-epidermal growth factor receptor antibody therapies. *Cancer Res*. 2007;67:2643-2648. doi:10.1158/0008-5472.CAN-06-4158
38. Karapetis CS, Khambata-Ford S, Jonker DJ, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. *N Engl J Med*. 2008;359:1757-1765. doi:10.1056/NEJMoa0804385
39. Van Cutsem E, Lenz HJ, Kohne CH, et al. Fluorouracil, leucovorin, and irinotecan plus cetuximab treatment and RAS mutations in colorectal cancer. *J Clin Oncol*. 2015;33:692-700. doi:10.1200/JCO.2014.59.4812
40. Ciardiello F, Normanno N, Maiello E, et al. Clinical activity of FOLFIRI plus cetuximab according to extended gene mutation status by next-generation sequencing: findings from the CAPRI-GOIM trial. *Ann Oncol*. 2014;25:1756-1761. doi:10.1093/annonc/mdu230
41. Ros J, Baraibar I, Martini G, et al. The evolving role of consensus molecular subtypes: a step beyond inpatient selection for

treatment of colorectal cancer. *Curr Treat Options Oncol.* 2021;22:113. doi:10.1007/s11864-021-00913-5

42. Irahara N, Baba Y, Nosho K, et al. NRAS mutations are rare in colorectal cancer. *Diagn Mol Pathol.* 2010;19:157-163. doi:10.1097/PDM.0b013e3181c93fd1
43. Nassar AH, Adib E, Kwiatkowski DJ. Distribution of $KRAS^{G12C}$ somatic mutations across race, sex, and cancer type. *N Engl J Med.* 2021;384:185-187. doi:10.1056/NEJMc2030638
44. Schirripa M, Nappo F, Cremolini C, et al. KRAS G12C metastatic colorectal cancer: specific features of a new emerging target population. *Clin Colorectal Cancer.* 2020;19:219-225. doi:10.1016/j.clcc.2020.04.009
45. Tol J, Nagtegaal ID, Punt CJ. BRAF mutation in metastatic colorectal cancer [erratum in: *N Engl J Med*. 2011;365:869]. *N Engl J Med.* 2009;361:98-99. doi:10.1056/NEJMc0904160
46. Roth AD, Tejpar S, Delorenzi M, et al. Prognostic role of KRAS and BRAF in stage II and III resected colon cancer: results of the translational study on the PETACC-3, EORTC 40993, SAKK 60-00 trial. *J Clin Oncol.* 2010;28:466-474. doi:10.1200/JCO.2009.23.3452
47. Cohen R, Liu H, Fiskum J, et al. BRAF V600E mutation in first-line metastatic colorectal cancer: an analysis of individual patient data from the ARCAD database. *J Natl Cancer Inst.* 2021;113:1386-1395. doi:10.1093/jnci/djab042
48. Seppala TT, Bohm JP, Friman M, et al. Combination of microsatellite instability and BRAF mutation status for subtyping colorectal cancer. *Br J Cancer.* 2015;112:1966-1975. doi:10.1038/bjc.2015.160
49. Lochhead P, Kuchiba A, Imamura Y, et al. Microsatellite instability and BRAF mutation testing in colorectal cancer prognostication. *J Natl Cancer Inst.* 2013;105:1151-1156. doi:10.1093/jnci/djt173
50. Fanelli GN, Dal Pozzo CA, Depetris I, et al. The heterogeneous clinical and pathological landscapes of metastatic Braf-mutated colorectal cancer. *Cancer Cell Int.* 2020;20:30. doi:10.1186/s12935-020-1117-2
51. 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. doi:10.1056/NEJMoa1908075
52. Barras D, Missiaglia E, Wirapati P, et al. BRAF V600E mutant colorectal cancer subtypes based on gene expression. *Clin Cancer Res.* 2017;23:104-115. doi:10.1158/1078-0432.CCR-16-0140
53. 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. doi:10.1200/JCO.2016.71.4394
54. Sepulveda AR, Hamilton SR, Allegra CJ, et al. Molecular biomarkers for the evaluation of colorectal cancer: guideline from the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and the American Society of Clinical Oncology. *J Clin Oncol.* 2017;35:1453-1486. doi:10.1200/JCO.2016.71.980
55. Van Cutsem E, Cervantes A, Adam R, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol.* 2016;27:1386-1422. doi:10.1093/annonc/mdw235
56. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol.* 2020;31:1491-1505. doi:10.1016/j.annonc.2020.07.014
57. Russo A, Incorvaia L, Del Re M, et al. The molecular profiling of solid tumors by liquid biopsy: a position paper of the AIOM-SIAPEC-IAP-SIBioC-SIC-SIF Italian Scientific Societies. *ESMO Open.* 2021;6:100164. doi:10.1016/j.esmoop.2021.100164
58. Normanno N, Esposito Abate R, Lambiase M, et al. RAS testing of liquid biopsy correlates with the outcome of metastatic colorectal cancer patients treated with first-line FOLFIRI plus cetuximab in the CAPRI-GOIM trial. *Ann Oncol.* 2018;29:112-118. doi:10.1093/annonc/mdx417
59. Khan KH, Cunningham D, Werner B, et al. Longitudinal liquid biopsy and mathematical modeling of clonal evolution forecast time to treatment failure in the PROSPECT-C phase II colorectal cancer clinical trial. *Cancer Discov.* 2018;8:1270-1285. doi:10.1158/2159-8290.CD-17-0891
60. Patelli G, Vaghi C, Tosi F, et al. Liquid biopsy for prognosis and treatment in metastatic colorectal cancer: circulating tumor cells vs circulating tumor DNA [erratum in: *Target Oncol*. 2021;16:413]. *Target Oncol.* 2021;16:309-324. doi:10.1007/s11523-021-00795-5
61. 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. doi:10.1002/cncr.31125
62. 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. doi:10.1158/2159-8290.CD-11-0109
63. Raghav K, Loree JM, Morris JS, et al. Validation of HER2 amplification as a predictive biomarker for anti-epidermal growth factor receptor antibody therapy in metastatic colorectal cancer. *JCO Precis Oncol.* 2019;3:1-13. doi:10.1200/PO.18.00226
64. 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. doi:10.1634/theoncologist.2018-0785
65. Bregni G, Sciallero S, Sobrero A. HER2 amplification and anti-EGFR sensitivity in advanced colorectal cancer. *JAMA Oncol.* 2019;5:605-606. doi:10.1001/jamaoncol.2018.7229
66. Pietrantonio F, Di Nicolantonio F, Schrock AB, et al. ALK, ROS1, and NTRK rearrangements in metastatic colorectal cancer. *J Natl Cancer Inst.* 2017;109:djx089. doi:10.1093/jnci/djx089
67. Xiao Y, Freeman GJ. The microsatellite instable subset of colorectal cancer is a particularly good candidate for checkpoint blockade immunotherapy. *Cancer Discov.* 2015;5:16-18. doi:10.1158/2159-8290.CD-14-1397
68. Llosa NJ, Cruise M, Tam A, et al. The vigorous immune microenvironment of microsatellite instable colon cancer is balanced by multiple counter-inhibitory checkpoints. *Cancer Discov.* 2015;5:43-51. doi:10.1158/2159-8290.CD-14-0863
69. Ciardiello D, Vitiello PP, Cardone C, et al. Immunotherapy of colorectal cancer: challenges for therapeutic efficacy. *Cancer Treat Rev.* 2019;76:22-32. doi:10.1016/j.ctrv.2019.04.003
70. Rayner E, van Gool IC, Palles C, et al. A panoply of errors: polymerase proofreading domain mutations in cancer. *Nat Rev Cancer.* 2016;16:71-81. doi:10.1038/nrc.2015.12
71. Domingo E, Freeman-Mills L, Rayner E, et al. Somatic POLE proofreading domain mutation, immune response, and prognosis in colorectal cancer: a retrospective, pooled biomarker study. *Lancet Gastroenterol Hepatol.* 2016;1:207-216. doi:10.1016/S2468-1253(16)30014-0

72. Dienstmann R, Vermeulen L, Guinney J, Kopetz S, Tejpar S, Tabernero J. Consensus molecular subtypes and the evolution of precision medicine in colorectal cancer [erratum in: *Nat Rev Cancer*. 2017;17:268]. *Nat Rev Cancer.* 2017;17:79-92. doi:10.1038/nrc.2016.126

73. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. *Nat Med*. 2015;21:1350-1356. doi:10.1038/nm.3967

74. Fessler E, Drost J, van Hooff SR, et al. TGFβ signaling directs serrated adenomas to the mesenchymal colorectal cancer subtype. *EMBO Mol Med*. 2016;8:745-760. doi:10.15252/emmm.201606184

75. Chang K, Willis JA, Reumers J, et al. Colorectal premalignancy is associated with consensus molecular subtypes 1 and 2. *Ann Oncol.* 2018;29:2061-2067. doi:10.1093/annonc/mdy337

76. Marisa L, Blum Y, Taieb J, et al. Intratumor CMS heterogeneity impacts patient prognosis in localized colon cancer. *Clin Cancer Res.* 2021;27:4768-4780. doi:10.1158/1078-0432.CCR-21-0529

77. Eide PW, Moosavi SH, Eilertsen IA, et al. Metastatic heterogeneity of the consensus molecular subtypes of colorectal cancer. *NPJ Genom Med.* 2021;6:59. doi:10.1038/s41525-021-00223-7

78. Martini G, Dienstmann R, Ros J, et al. Molecular subtypes and the evolution of treatment management in metastatic colorectal cancer. *Ther Adv Med Oncol.* 2020;12:1758835920936089. doi:10.1177/1758835920936089

79. 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.

80. Rabeneck L, Chiu HM, Senore C. International perspective on the burden of colorectal cancer and public health effects. *Gastroenterology.* 2020;158:447-452.

81. Heidelberger C, Chaudhuri NK, Danneberg P, et al. Fluorinated pyrimidines, a new class of tumour-inhibitory compounds. *Nature.* 1957;179:663-666.

82. Rustum YM, Trave F, Zakrzewski SF, et al. Biochemical and pharmacologic basis for potentiation of 5-fluorouracil action by leucovorin. *NCI Monogr.* 1987;5:165-170.

83. 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.* 2004;22:23-30.

84. 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.

85. De Falco V, Napolitano S, Rosello S, et al. How we treat metastatic colorectal cancer. *ESMO Open.* 2020;4(suppl 2):e000813.

86. Riihimaki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. *Sci Rep.* 2016;6:29765.

87. Datta J, Narayan RR, Kemeny NE, D'Angelica MI. Role of hepatic artery infusion chemotherapy in treatment of initially unresectable colorectal liver metastases. a review. *JAMA Surg.* 2019;154:768-776. doi:10.1001/jamasurg.2019.1694

88. Saito T, Niida A, Uchi R, et al. A temporal shift of the evolutionary principle shaping intratumor heterogeneity in colorectal cancer. *Nat Commun.* 2018;9:2884.

89. 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. doi:10.1056/NEJMoa1403108

90. Kabbinavar F, Hurwitz HI, Fehrenbacher L, et al. Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. *J Clin Oncol.* 2003;21:60-66.

91. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004;350:2335-2342.

92. Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study [erratum in: *J Clin Oncol*. 2009;27:653]. *J Clin Oncol.* 2008;26:2013-2019. doi:10.1200/JCO.2007.14.9930

93. 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.

94. Hegewisch-Becker S, Graeven U, Lerchenmuller 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 [erratum in: *Lancet Oncol*. 2015;16:e589]. *Lancet Oncol*. 2015;16:1355-1369. doi:10.1016/S1470-2045(15)00042-X

95. Simkens LH, van Tinteren H, 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. doi:10.1016/S0140-6736(14)62004-3

96. Cremolini C, Marmorino F, Loupakis F, et al. TRIBE-2: a phase III, randomized, open-label, strategy trial in unresectable metastatic colorectal cancer patients by the GONO group. *BMC Cancer.* 2017;17:408. doi:10.1186/s12885-017-3360-z

97. 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

98. 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. doi:10.1016/S1470-2045(12)70477-1

99. Van Cutsem E, Tabernero J, Lakomy R, et al. Addition of aflibercept to fluorouracil, leucovorin, and irinotecan improves survival in a phase III randomized trial in patients with metastatic colorectal cancer previously treated with an oxaliplatin-based regimen. *J Clin Oncol.* 2012;30:3499-3506. doi:10.1200/JCO.2012.42.8201

100. 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 [erratum in: *Lancet Oncol*. 2015;16:e262]. *Lancet Oncol.* 2015;16:499-508. doi:10.1016/S1470-2045(15)70127-0

101. Kemeny NE, Jarnagin WR, Capanu M, et al. Randomized phase II trial of adjuvant hepatic arterial infusion and systemic chemotherapy with or without bevacizumab in patients with resected hepatic metastases from colorectal cancer. *J Clin Oncol.* 2011;29:884-889. doi:10.1200/JCO.2010.32.5977

102. Grothey A, Van Cutsem E, Sobrero A, et al. Regorafenib monotherapy for previously treated metastatic colorectal cancer (CORRECT): an international, multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet.* 2013;381:303-312. doi:10.1016/S0140-6736(12)61900-X

103. Van Cutsem E, Martinelli E, Cascinu S, et al. Regorafenib for patients with metastatic colorectal cancer who progressed after standard therapy: results of the large, single-arm, open-label phase IIIb CONSIGN study. *Oncologist*. 2019;24:185-192. doi:10.1634/theoncologist.2018-0072

104. Martini G, Ciardiello D, Vitiello PP, et al. Resistance to anti-epidermal growth factor receptor in metastatic colorectal cancer: what does still need to be addressed? *Cancer Treat Rev*. 2020;86:102023.

105. Parseghian CM, Napolitano S, Loree JM, Kopetz S. Mechanisms of innate and acquired resistance to anti-EGFR therapy: a review of current knowledge with a focus on a rechallenge therapies. *Clin Cancer Res*. 2019;25:6899-6808.

106. Jonker DJ, O'Callaghan CJ, Karapetis CS, et al. Cetuximab for the treatment of colorectal cancer. *N Engl J Med*. 2007;357:2040-2048. doi:10.1056/NEJMoa071834

107. Loree JM, Dowers A, Tu D, et al. Expanded low allele frequency RAS and BRAF V600E testing in metastatic colorectal cancer as predictive biomarkers for cetuximab in the randomized CO.17 trial. *Clin Cancer Res*. 2021;27:52-59. doi:10.1158/1078-0432.CCR-20-2710

108. Bokemeyer C, Bondarenko I, Hartmann JT, et al. Efficacy according to biomarker status of cetuximab plus FOLFOX-4 as first-line treatment for metastatic colorectal cancer: the OPUS study. *Ann Oncol*. 2011;22:1535-1546. doi:10.1093/annonc/mdq632

109. 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. doi:10.1016/S0140-6736(11)60613-2

110. Qin S, Li J, Wang L, Xu J, et al. Efficacy and tolerability of first-line cetuximab plus leucovorin, fluorouracil, and oxaliplatin (FOLFOX-4) Versus FOLFOX-4 in patients with RAS wild-type metastatic colorectal cancer: the open-label, randomized, phase III TAILOR trial. *J Clin Oncol*. 2018;36:3031-3039. doi:10.1200/JCO.2018.78.3183

111. Venook AP, Niedzwiecki D, Lenz H, et al. Effect of first-line chemotherapy combined with cetuximab or bevacizumab on overall survival in patients with KRAS wild-type advanced or metastatic colorectal cancer: a randomized clinical trial. *JAMA*. 2017;317:2392-2401. doi:10.1001/jama.2017.7105

112. 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. doi:10.1016/S1470-2045(14)70330-4

113. 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. doi:10.1200/JCO.2007.13.1193

114. Ciardiello F, Normanno N, Martinelli E, et al. Cetuximab continuation after first progression in metastatic colorectal cancer (CAPRI-GOIM): a randomized phase II trial of FOLFOX plus cetuximab versus FOLFOX. *Ann Oncol*. 2016;27:1055-1061. doi:10.1093/annonc/mdw136

115. Cremolini C, Antoniotti C, Lonardi S, et al. Activity and safety of cetuximab plus modified FOLFOXIRI followed by maintenance with cetuximab or bevacizumab for RAS and BRAF wild-type metastatic colorectal cancer: a randomized phase 2 clinical trial. *JAMA Oncol*. 2018;4:529-536. doi:10.1001/jamaoncol.2017.5314

116. Arnold D, Lueza B, Douillard JY, 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.

117. Martini G, Cardone C, Vitiello PP, et al. EphA2 is a predictive biomarker of resistance and a potential therapeutic target for improving antiepidermal growth factor receptor therapy in colorectal cancer. *Mol Cancer Ther*. 2019;18:845-855. doi:10.1158/1535-7163.MCT-18-0539

118. Ciardiello D, Blauensteiner B, Matrone N, et al. Dual inhibition of TGFβ and AXL as a novel therapy for human colorectal adenocarcinoma with mesenchymal phenotype. *Med Oncol*. 2021;38:24. doi:10.1007/s12032-021-01464-3

119. Troiani T, Martinelli E, Napolitano S, et al. Increased TGF-α as a mechanism of acquired resistance to the anti-EGFR inhibitor cetuximab through EGFR-MET interaction and activation of MET signaling in colon cancer cells. *Clin Cancer Res*. 2013;19:6751-6765. doi:10.1158/1078-0432.CCR-13-0423

120. Day FL, Jorissen RN, Lipton L, et al. PIK3CA and PTEN gene and exon mutation-specific clinicopathologic and molecular associations in colorectal cancer. *Clin Cancer Res*. 2013;19:3285-3296. doi:10.1158/1078-0432.CCR-12-3614

121. Aderka D, Stintzing S, Heinemann V. Explaining the unexplainable: discrepancies in results from the CALGB/SWOG 80405 and FIRE-3 studies. *Lancet Oncol*. 2019;20:e274-e283. doi:10.1016/S1470-2045(19)30172-X

122. Lenz HJ, Ou FS, Venook AP, et al. Impact of consensus molecular subtype on survival in patients with metastatic colorectal cancer: results from CALGB/SWOG 80405 (Alliance) [errata in: *J Clin Oncol*. 2020;38:656]. *J Clin Oncol*. 2019;37:1876-1885.

123. Stintzing S, Wirapati P, Lenz HJ, et al. Consensus molecular subgroups (CMS) of colorectal cancer (CRC) and first-line efficacy of FOLFIRI plus cetuximab or bevacizumab in the FIRE3 (AIO KRK-0306) trial. *Ann Oncol*. 2019;30:1796-1803. doi:10.1093/annonc/mdz387

124. Misale S, Di Nicolantonio F, Sartore-Bianchi A, Siena S, Bardelli A. Resistance to anti-EGFR therapy in colorectal cancer: from heterogeneity to convergent evolution. *Cancer Discov*. 2014;4:1269-1280.

125. Parseghian CM, Loree JM, Morris VK, et al. Anti-EGFR resistant clones decay exponentially after progression: implications for anti-EGFR re-challenge. *Ann Oncol*. 2019;30:243-249.

126. Santini D, Vincenzi B, Addeo R, et al. Cetuximab rechallenge in metastatic colorectal cancer patients: how to come away from acquired resistance [erratum in: *Ann Oncol*. 2017;28:2906]? *Ann Oncol*. 2017;23:2313-2318.

127. Cremolini C, Rossini D, Dell'Aquila E, et al. Rechallenge for patients with RAS and BRAF wild-type metastatic colorectal cancer with acquired resistance to first-line cetuximab and irinotecan: a phase 2 single-arm clinical trial. *JAMA Oncol*. 2019;5:343-350.

128. Sunakawa Y, Nakamura M, Ishizaki M, et al. RAS mutations in circulating tumor DNA and clinical outcomes of rechallenge treatment with anti-EGFR antibodies in patients with metastatic colorectal cancer. *JCO Precis Oncol*. 2020;4:898-911.

129. Martinelli E, Martini G, Famiglietti V, et al. Cetuximab rechallenge plus avelumab in pretreated patients with RAS wild-type metastatic colorectal cancer: the phase 2 single-arm clinical CAVE trial. *JAMA Oncol*. 2021;7:1529.

130. Masuishi T, Tsuji A, Kotaka M, et al. Phase 2 study of irinotecan plus cetuximab rechallenge as third-line treatment in KRAS wild-type metastatic colorectal cancer: JACCRO CC-08. *Br J Cancer*. 2020;123:1490-1495.

131. Liu X, George GC, Tsimberidou AM, et al. Retreatment with anti-EGFR based therapies in metastatic colorectal cancer: impact of intervening time interval and prior anti-EGFR response. *BMC Cancer*. 2015;15:713.

132. Tanioka H, Asano M, Yoshida R, et al. Cetuximab retreatment in patients with metastatic colorectal cancer who exhibited a clinical benefit in response to prior cetuximab: a retrospective study. *Oncol Lett*. 2018;16:3674-3680.

133. Rossini D, Germani MM, Pagani F, et al. Retreatment with anti-EGFR antibodies in metastatic colorectal cancer patients: a multi-institutional analysis. *Clin Colorectal Cancer*. 2020;19:191-199.e6.

134. Karani A, Felismino TC, Diniz L, Macedo MP, Silva VSE, Mello CA. Is there a role for rechallenge and reintroduction of anti-EGFR plus chemotherapy in later lines of therapy for metastatic colorectal carcinoma? *A retrospective analysis. Ecancermedicalscience.* 2020;14:1069. doi:10.3332/ecancer.2020.1069

135. Chong LC, Hardingham JE, Townsend AR, et al. Rechallenge with anti-EGFR therapy in metastatic colorectal cancer (mCRC): results from South Australia mCRC Registry. *Target Oncol.* 2020;15:751-757.

136. 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.

137. Yaeger R, Cercek A, O'Reilly EM, et al. Pilot trial of combined BRAF and EGFR inhibition in BRAF-mutant metastatic colorectal cancer patients. *Clin Cancer Res.* 2015;21:1313-1320.

138. Corcoran RB, Atreya CE, Falchook GS, et al. Combined BRAF and MEK inhibition with dabrafenib and trametinib in BRAF V600-mutant colorectal cancer. *J Clin Oncol.* 2015;33:4023-4031. doi:10.1200/JCO.2015.63.2471

139. Corcoran RB, Andre T, Atreya CE, et al. Combined BRAF, EGFR, and MEK inhibition in patients with $BRAV^{V600E}$-mutant colorectal cancer. *Cancer Discov.* 2018;8:428-443. doi:10.1158/2159-8290.CD-17-1226

140. van Geel RMJM, Tabenero 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.

141. Kopetz S, McDonough SL, Lenz HJ, et al. Randomized trial of irinotecan and cetuximab with or without vemurafenib in BRAF-mutant metastatic colorectal cancer (SWOG S1406) [abstract]. *J Clin Oncol.* 2017;35(15 suppl):3505.

142. 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. doi:10.1038/nature10868

143. 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 [abstract]. *Ann Oncol.* 2020;31(suppl 3):S242-S243.

144. Tabernero J, Grothey A, Van Cutsem E, et al. Encorafenib plus cetuximab as a new standard of care for previously treated BRAF V600E-mutant metastatic colorectal cancer: updated survival results and subgroup analyses from the BEACON study. *J Clin Oncol.* 2021;39:273-284. doi:10.1200/JCO.20.02088

145. Kopetz S, Grothey A, Rona Yaeger, et al. BREAKWATER: randomized phase 3 study of encorafenib (enco) + cetuximab (cetux) ± chemotherapy for first-line (1L) treatment (tx) of BRAF V600E-mutant (BRAFV600E) metastatic colorectal cancer (mCRC) [abstract]. *J Clin Oncol.* 2021;39(15 suppl):TPS3619.

146. 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.

147. 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;5:3000911.

148. Meric-Bernstam F, Hurwitz H, Singh Raghav KP, et al. Pertuzumab plus trastuzumab for HER2-amplified metastatic colorectal cancer (MyPathway): an updated report from a multicentre, open-label, phase 2a, multiple basket study. *Lancet Oncol.* 2019;20:518-530.

149. Strickler JH, Zemla T, Ou FS, et al. 527PD Trastuzumab and tucatinib for the treatment of HER2 amplified metastatic colorectal cancer (mCRC): initial results from the MOUNTAINEER trial [abstract]. *Ann Oncol.* 2019;30(suppl 5):v200.

150. Nakamura Y, Okamoto W, Kato T, et al. 526PD TRIUMPH: primary efficacy of a phase II trial of trastuzumab (T) and pertuzumab (P) in patients (pts) with metastatic colorectal cancer (mCRC) with HER2 (ERBB2) amplification (amp) in tumour tissue or circulating tumour DNA (ctDNA): a GOZILA sub-study [abstract]. *Ann Oncol.* 2019;30(suppl 5):v199-v200.

151. 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. doi:10.1016/S1470-2045(21)00086-3

152. Fakih MG, Kopetz S, Kuboki Y, et al. Sotorasib for previously treated colorectal cancers with KRASG12C mutation (CodeBreaK100): a prespecified analysis of a single-arm, phase 2 trial. *Lancet Oncol.* 2022;23:115-124. doi:10.1016/S1470-2045(21)00605-7

153. Adagrasib data create buzz at ESMO. *Cancer Discov*. 2021;11:2664. doi:10.1158/2159-8290.CD-NB2021-0385

154. Hong DS, Fakih MG, Strickler JH, et al. $KRAS^{G12C}$ inhibition with sotorasib in advanced solid tumors. *N Engl J Med.* 2020;383:1207-1217. doi:10.1056/NEJMoa1917239

155. 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(suppl 5):S121294. doi:10.1016/annonc/annonc741

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

157. 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. doi:10.1016/S1470-2045(19)30691-6

158. Ricciuti B, Genova C, Crino L, Libra M, Leonardi GC. Antitumor activity of larotrectinib in tumors harboring NTRK gene fusions: a short review on the current evidence [erratum in: *Onco Targets Ther*. 2020;13:3975]. *Onco Targets Ther.* 2019;12:3171-3179. doi:10.2147/OTT.S177051

159. Mayer RJ, Van Cutsem E, Falcone A, et al; RECOURSE Study Group. Randomized trial of TAS-102 for refractory metastatic colorectal cancer. *N Engl J Med.* 2015;372:1909-1919.

160. Vitale P, Zanaletti N, Famiglietti V, et al. Retrospective study of regorafenib versus TAS-102 efficacy and safety in chemorefractory metastatic

colorectal cancer (mCRC) patients: a multi-institution real life clinical data. *Clin Colorectal Cancer.* 2021;20:227-235.

161. Li GM. Mechanisms and functions of DNA mismatch repair. *Cell Res.* 2008:18;85-98.

162. 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.

163. Arrichiello G, Poliero L, Borrelli C, et al. Immunotherapy in colorectal cancer: is the long-awaited revolution finally happening? *Cancer Treat Res Commun.* 2021;28:100442. doi:10.1016/j.ctarc.2021.100442

164. 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.

165. Kamble S, Kopetz S, Andre T. 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.

166. 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. doi:10.1056/NEJMoa1500596

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

168. Shitara K, Van Cutsem E, Bang Y, et al. Efficacy and safety of pembrolizumab or pembrolizumab plus chemotherapy vs chemotherapy alone for patients with first-line, advanced gastric cancer: the KEYNOTE-062 phase 3 randomized clinical trial. *JAMA Oncol.* 2020;6:1571-1580. doi:10.1001/jamaoncol.2020.3370

169. Lenz HJ, Van Cutsem E, Luisa Limon M, 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.* 2022;40:161-170. doi:10.1200/JCO.21.01015

170. 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.

171. Chen EX, Jonker DJ, Loree J, et al. Effect of combined immune checkpoint inhibition vs. best supportive care alone in patients with advanced colorectal cancer. *JAMA Oncol.* 2020;6:831-838.

172. 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. doi:10.1200/JCO.19.03296

173. Cremolini C, Rossini D, Antoniotti C, et al. LBA20 FOLFOXIRI plus bevacizumab (bev) plus atezolizumab (atezo) versus FOLFOXIRI plus bev as first-line treatment of unresectable metastatic colorectal cancer (mCRC) patients: results of the phase II randomized AtezoTRIBE study by GONO. *Ann Oncol.* 2021;32(suppl 5):S1294-S1295.

174. Pietrantonio F, Morano F, Lonardi S, et al. 383O MAYA trial: temozolomide (TMZ) priming followed by combination with low-dose ipilimumab and nivolumab in patients with microsatellite stable (MSS), MGMT silenced metastatic colorectal cancer (mCRC). *Ann Oncol.* 2021;32(suppl 5):S530-S532.

175. Wang W, Wu L, Zhang J, Wu H, Han E, Guo Q. Chemoimmunotherapy by combining oxaliplatin with immune checkpoint blockades reduced tumor burden in colorectal cancer animal model. *Biochem Biophys Res Commun.* 2017;487:1-7.

176. Vincent J, Mignot G, Chalmin F, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. *Cancer Res.* 2010;70:3052-3061.

177. Kanterman J, Sade-Feldman M, Biton M, et al. Adverse immunoregulatory effects of 5FU and CPT11 chemotherapy on myeloid-derived suppressor cells and colorectal cancer outcomes. *Cancer Res.* 2014;74:6022-6035.

178. Osada T, Chong G, Tansik R, et al. The effect of anti-VEGF therapy on immature myeloid cell and dendritic cells in cancer patients. *Cancer Immunol Immunother.* 2008;57:1115-1124. doi:10.1007/s00262-007-0441-x

179. Limagne E, Euvrard R, Thibaudin M, et al. Accumulation of MDSC and Th17 cells in patients with metastatic colorectal cancer predicts the efficacy of a FOLFOX-bevacizumab drug treatment regimen. *Cancer Res.* 2016:76;5241-5252.

180. Garralda E, Sukari A, Lakhani NJ, et al. A phase 1 first-in-human study of the anti-LAG-3 antibody MK4280 (favezelimab) plus pembrolizumab in previously treated, advanced microsatellite stable colorectal cancer [abstract]. *J Clin Oncol.* 2021;39(suppl 15):3584.

181. Calegari MA, Inno A, Monterisi S, et al. A phase 2 study of temozolomide in pretreated metastatic colorectal cancer with MGMT promoter methylation. *Br J Cancer.* 2017;116:1279-1286.

182. Grothey A, Tabernero J, Arnold D, et al. Fluoropyrimidine (FP) + bevacizumab (BEV) + atezolizumab vs. FP/BEV in BRAFwt metastatic colorectal cancer (mCRC): findings from cohort 2 of MODUL—a multicentre, randomized trial of biomarker-driven maintenance treatment following first-line induction therapy. *Ann Oncol.* 2018;29(suppl 8):VIII714-VIII715.

183. Doleschel D, Hoff S, Koletnik S, et al. Regorafenib enhances anti-PD1 immunotherapy efficacy in murine colorectal cancers and their combination prevents tumor regrowth. *J Exp Clin Cancer Res.* 2021;40:288. doi:10.1186/s13046-021-02043-0

184. Wu RY, Kong PF, Xia LP, et al. Regorafenib promotes antitumor immunity via inhibiting PD-L1 and IDO1 expression in melanoma. *Clin Cancer Res.* 2019;25:4530-4541. doi:10.1158/1078-0432.CCR-18-2840

185. Abou-Elkacem L, Arns S, Brix G, et al. Regorafenib inhibits growth, angiogenesis, and metastasis in a highly aggressive, orthotopic colon cancer model. *Mol Cancer Ther.* 2013;12:1322-1331.

186. Fakih M, Raghav KPS, Chang DZ, et al. Single-arm, phase 2 study of regorafenib plus nivolumab in patients with mismatch repair-proficient (pMMR)/microsatellite stable (MSS) colorectal cancer (CRC) [abstract]. *J Clin Oncol.* 2021;39(suppl 15):3560. doi:10.1200/JCO.2021.39.15_suppl.3560

187. Cousin S, Cantarel C, Guegan JP, 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.

188. Fasano M, Della Corte CM, Di Liello R, et al. Induction of natural killer antibody-dependent cell cytotoxicity and of clinical activity of cetuximab plus avelumab in non-small cell lung cancer. *ESMO Open.* 2020;5:e000753.

189. Ciardiello D, Famiglietti V, Napolitano S, et al. Skin toxicity as predictor of survival in refractory patients with RAS wild-type metastatic colorectal cancer treated with cetuximab and avelumab (CAVE) as rechallenge strategy. *Cancers (Basel).* 2021;13:5715. doi:10.3390/cancers13225715

190. Ciardiello D, Famiglietti V, Napolitano S, et al. Final results of the CAVE trial in RAS wild type metastatic colorectal cancer patients treated with cetuximab plus avelumab as rechallenge therapy: neutrophil to lymphocyte ratio predicts survival. *Clin Colorectal Cancer.* Published online January 16, 2022. doi:10.1016/j.clcc.2022.01.005

191. Sardo E, Napolitano S, Della Corte CM, et al Multi-omic approaches in colorectal cancer beyond genomic data. *J Pers Med.* 2022;12:128. doi:10.3390/jpm12020128

192. Wu C, Hsu CW, Chen CD, et al. Candidate serological biomarkers for cancer identified from the secretomes of 23 cancer cell lines and the Human Protein Atlas. *Mol Cell Proteomics.* 2010;9:1100-1117.

193. Boisvert FM, Lam YW, Lamont D, Lamond AO. Quantitative proteomics analysis of subcellular proteome localization and changes induced by DNA damage. *Mol Cell Proteomics.* 2010;9:457-470.

194. Fijneman RJ, De Wit M, Pourghiasian M, et al. Proximal fluid proteome profiling of mouse colon tumors reveals biomarkers for early diagnosis of human colorectal cancer. *Clin Cancer Res.* 2012;18:2613-2624.

195. Scott AJ, Alexander JL, Merrifield CA, et al. International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis. *Gut.* 2019;68:1624-1632.

196. Hanahan D. Hallmarks of cancer: new dimensions. *Cancer Discov.* 2022;12:31-46. doi:10.1158/2159-8290.CD-21-1059

197. Thomas S, Izard J, Walsh E, et al. The host microbiome regulates and maintains human health: a primer and perspective for non-microbiologists. *Cancer Res.* 2017;77:1783-1812. doi:10.1158/0008-5472.CAN-16-2929

198. Janney A, Powrie F, Mann EH. Host-microbiota maladaptation in colorectal cancer. *Nature.* 2020;585:509-517. doi:10.1038/s41586-020-2729-3

199. Paulos CM, Wrzesinski C, Kaiser A, et al. Microbial translocation augments the function of adoptively transferred self/tumor-specific CD8+ T cells via TLR4 signaling. *J Clin Invest.* 2007;117:2197-2204.

200. Routy B, Le Chatelier E, Derosa L, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science.* 2018;359:91-97.

201. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. *Nat Rev Gastroenterol Hepatol.* 2017;14:356-365.

202. Kespohl M, Vachharajani N, Luu M, et al. The microbial metabolite butyrate induces expression of Th1-associated factors in cD4+ T cells. *Front Immunol.* 2017;8:1036.

203. Zhang SL, Mao YQ, Zhang ZY, et al. Pectin supplement significantly enhanced the anti-PD-1 efficacy in tumor-bearing mice humanized with gut microbiota from patients with colorectal cancer. *Theranostics.* 2021;11:4155-4170.

204. Arsian E, Aksoy T, Gursu RU, Dursun N, Cakar E, Cermik TF. The prognostic value of 18F-FDG PET/CT and KRAS mutation in colorectal cancers. *Mol Imaging Radionucl Ther.* 2020;29:17-24.

205. Xu Y, Xu Q, Ma Y, et al. Characterizing MRI features of rectal cancers with different KRAS status. *BMC Cancer.* 2019;19:1111. doi:10.1186/s12885-019-6341-6

206. Taguchi N, Oda S, Yokota Y, et al. CT texture analysis for the prediction of KRAS mutation status in colorectal cancer via a machine learning approach. *Eur J Radiol.* 2019;118:38-43.

207. Cao Y, Zhang G, Zhang J, et al. Predicting microsatellite instability status in colorectal cancer based on triphasic enhanced computed tomography radiomics signatures: a multicenter study. *Front Oncol.* 2021;11:687771. doi:10.3389/fonc.2021.687771

208. Douillard JY, Siena S, Cassidy J, et al. Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: the PRIME study. *J Clin Oncol.* 2010;28:4697-4705. doi:10.1200/JCO.2009.27.4860

209. Douillard JY, Oliner KS, Siena S, et al. Panitumumab-FOLFOX4 treatment and RAS mutations in colorectal cancer. *N Engl J Med.* 2013;369:1023-1034. doi:10.1056/NEJMoa1305275

210. 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. doi:10.1200/JCO.2013.53.2473

211. Modest DP, Martens UM, Riera-Knorrenschild J, et al. FOLFOXIRI plus panitumumab as first-line treatment of RAS wild-type metastatic colorectal cancer: the randomized, open-label, phase II VOLFI study (AIO KRK0109). *J Clin Oncol.* 2019;37:3401-3411. doi:10.1200/JCO.19.01340

212. Pietrantonio F, Morano F, Corallo S, et al. Maintenance therapy with panitumumab alone vs panitumumab plus fluorouracil-leucovorin in patients with RAS wild-type metastatic colorectal cancer: a phase 2 randomized clinical trial. *JAMA Oncol.* 2019;5:1268-1275. doi:10.1001/jamaoncol.2019.1467

213. Seymour MT, Brown SR, Middleton G, et al. Panitumumab and irinotecan versus irinotecan alone for patients with KRAS wild-type, fluorouracil-resistant advanced colorectal cancer (PICCOLO): a prospectively stratified randomised trial. *Lancet Oncol.* 2013;14:749-759. doi:10.1016/S1470-2045(13)70163-3

214. 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. doi:10.1200/JCO.2009.27.6055

215. Peeters M, Price TJ, Cervantes A, et al. Final results from a randomized phase 3 study of FOLFIRI +/− panitumumab for second-line treatment of metastatic colorectal cancer [erratum in: *Ann Oncol.* 2014;25:757]. *Ann Oncol.* 2014;25:107-116. doi:10.1093/annonc/mdt523

216. Carrato A, Abad A, Massuti B, et al; Spanish Cooperative Group for the Treatment of Digestive Tumours (TTD). First-line panitumumab plus FOLFOX4 or FOLFIRI in colorectal cancer with multiple or unresectable liver metastases: a randomised, phase II trial (PLANET-TTD). *Eur J Cancer.* 2017;81:191-202. doi:10.1016/j.ejca.2017.04.024

217. Modest DP, Karthaus M, Fruehauf S, et al. Panitumumab plus fluorouracil and folinic acid versus fluorouracil and folinic acid alone as maintenance therapy in RAS wild-type metastatic colorectal cancer: the randomized PANAMA trial (AIO KRK 0212). *J Clin Oncol.* 2022;40:72-82. doi:10.1200/JCO.21.01332

218. Price TJ, Peeters M, Kim TW, et al. Panitumumab versus cetuximab in patients with chemotherapy-refractory wild-type KRAS exon 2 metastatic colorectal cancer (ASPECCT): a randomised, multicentre, open-label, non-inferiority phase 3 study. *Lancet Oncol.* 2014;15:569-579. doi:10.1016/S1470-2045(14)70118-4