Chapter

Landscape of Current Targeted Therapies for Advanced Colorectal Cancer

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Abstract

Colorectal cancer (CRC) is one of the most frequent and lethal cancer types worldwide. While surgery with chemotherapy and radiotherapy remains the only curative approach for localized CRC, for metastatic disease the therapeutic landscape has significantly evolved over the last years. Development and approval of novel targeted therapies, such as monoclonal antibodies against EGFR and VEGF, have significantly increased the median survival of patients with metastatic disease, with some trials reporting a benefit over 40 months. Increasing accessibility of high throughput sequencing has unraveled several new therapeutic targets. Actionable alterations, such as HER2 overexpression, BRAF mutations, and NTRK fusions, are currently available in metastatic disease, providing significant therapeutic opportunities for these patients, while new emerging agents, as immune checkpoint inhibitors, promise better treatment options in the near future. In this chapter, an overview of established and future CRC targeted therapies in the clinical setting is provided, as well as their mechanism of action, limitations, and future applicability.

Keywords: EGFR, immunotherapy, metastatic colorectal cancer, targeted therapy, VEGF

1. Introduction

Colorectal cancer is the third most common cancer worldwide and remains an important cause of death. CRC diagnosis and treatment require a multidisciplinary approach, and in stage IV disease combination chemotherapy (CT) and regional multimodality treatments – like metastasectomy and other local treatments – are increasingly used. Systemic therapy has evolved over the past few decades, with the emergence of combination CT and targeted agents (**Figure 1**).

In the present review, genomic and tumor microenvironment alterations driving treatment selection are discussed.

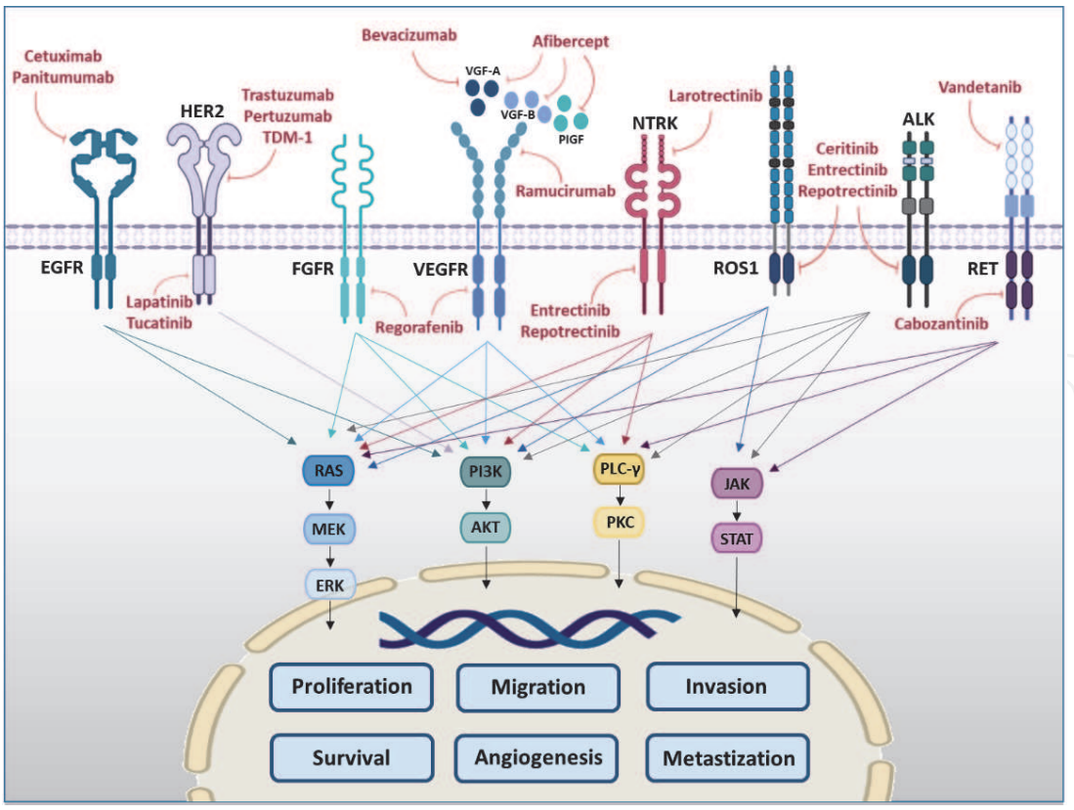

Figure 1.
Targeted therapies that have been approved or are currently under investigation for advanced colorectal cancer.

1.1 Historical background

Metastatic CRC (mCRC) presents with synchronous metastatic disease at initial diagnosis in 20% of cases, with 50–60% of patients developing metachronous metastases. Approximately 56% of patients with CRC will ultimately die from their cancer [1]. The cornerstone of CRC treatment for 20 years has been fluoropyrimidine-based CT doublets, with either irinotecan (FOLFIRI or CAPIRI) or oxaliplatin (FOLFOX or CAPOX) in the first- and second-line settings [2].

In the past two decades, remarkable progress has been achieved in mCRC treatment with the introduction of molecular targeted agents (**Figure 2**). Today, the median overall survival (OS) for these patients in phase III trials is approximately 30 months, more than doubling that of 20 years ago [3]. Simultaneously, mortality has declined, what is attributed to earlier diagnosis (due to screening tests) and improved treatment options, including new systemic CT agents and biologic agents targeting specific pathways [1].

More recently, consensus molecular subtypes (CMS) defined by gene expression profiling have identified biologically different CRC subtypes, which seem to have a prognostic and predictive value. However, CMS subtyping is not a standard test with therapeutic application at present, being more relevant in the research field [2].

2. EGFR pathway

New targeted therapies against the epidermal growth factor receptor (EGFR) had an impressive impact on mCRC prognosis, with an actual median OS over 30 months (varying according to therapeutics options) [4–6].

As part of the ErbB tyrosine kinase family, EGFR is a transmembrane receptor and its activation by extracellular ligands stimulates downstream pathways, such as

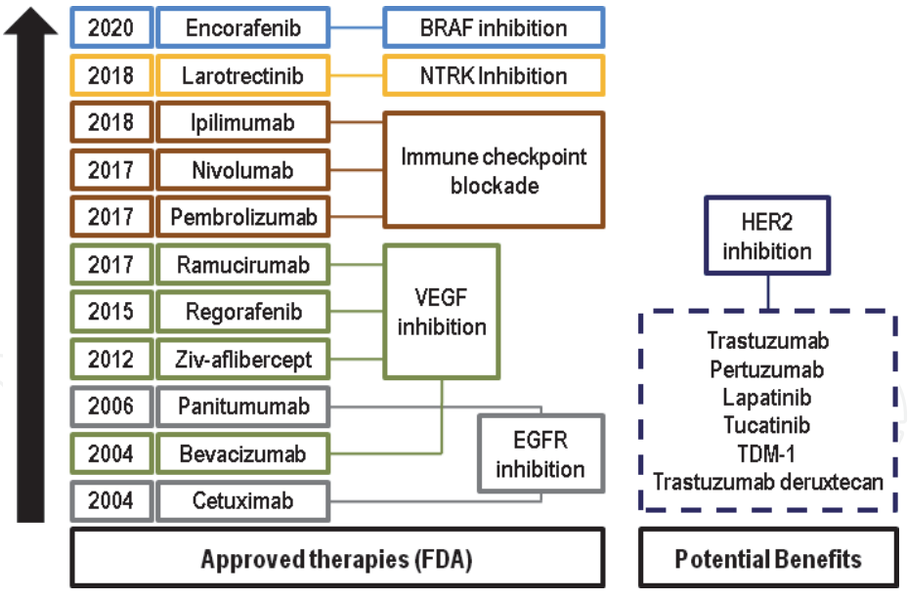

Figure 2.
Timeline of development of targeted therapies in colon cancer.

RAS–RAF–MEK-MAPK, PIK3CA-AKT, the SRC family kinases, PLCγ-PKC, and JAK/STATs, inducing proliferation, migration, invasion, survival, and angiogenesis [6, 7]. Thus, EGFR is an important factor in tumor development and progression, being expressed in various cancers and in 60–80% of CRCs [8].

Target therapy against EGFR is now a standard of care in RAS wild-type mCRC. Two monoclonal antibodies (mAbs) are approved: cetuximab (human-mouse chimeric mAb) and panitumumab (fully human mAb). By recognizing and binding to the extracellular domain of the EGFR receptor, these mAbs prevent binding of other extracellular ligands and subsequent receptor internalization and degradation, thus inhibiting and blocking downstream pathways and signaling [9]. Tumor RAS mutational status predicts efficacy of anti-EGFR agents in mCRC patients, with RAS mutations being a well-established negative predictive biomarker for patient selection [10].

2.1 Clinical trials

Several phase II and III clinical trials have established the efficacy of cetuximab and panitumumab, either in monotherapy or in association with CT, in terms of progression-free survival (PFS), OS, and overall response rate (RR), while maintaining quality of life (**Table 1**) [6, 11–13].

2.1.1 First-line setting

The PRIME trial, a randomized phase III trial investigating the addition of panitumumab to FOLFOX4 as first-line therapy in RAS wild-type mCRC, showed a 2- and 6-month PFS and OS benefit, respectively, with the combination. Regarding safety, known EGFR inhibition adverse events (AE) were more frequently observed with panitumumab, including skin toxicity and diarrhea (36% vs. 2% and 18% vs. 9% in panitumumab and placebo arms, respectively) [11].

The randomized phase II PEAK trial compared the efficacy and safety of mFOLFOX6 plus panitumumab with mFOLFOX plus bevacizumab (an anti- vascular endothelial growth factor [VEGF] mAb) as first-line therapy in RAS wild-type mCRC. The study primary endpoint was met, with panitumumab showing a 3.5-month PFS increase compared with bevacizumab. An OS improvement was also

observed, although not statistically significant [14]. Rivera et al. and Stintzing S et al. also demonstrated that early tumor shrinkage in an important and early predictor of treatment sensitivity and deep tumor response correlates with OS [15, 16].

The open-label phase II PLANET-TTD trial compared panitumumab with two different CT regimens (FOLFOX 4 and FOLFIRI) as first-line treatment of RAS wild-type mCRC, but no significant efficacy differences were observed between the two regimens [17].

The 314 trial, a single-arm phase II study evaluating first-line panitumumab plus FOLFIRI in mCRC patients, confirmed the impact of KRAS exon 2 status in being a negative predictor of efficacy in mutant patients. In a total of 154 patients, 59% had KRAS wild-type tumors. RR and median duration of response (DoR) were higher in the KRAS wild-type group. Additionally, more patients in the wild-type group underwent R0 resection (8% vs. 5%), and a PFS benefit was also observed in this group (8.9 vs. 7.2 months) [18].

In the COIN trial, cetuximab was added to oxaliplatin-containing CT (FOLFOX or CAPOX) in first-line setting of mCRC. In patients with KRAS wild-type tumors, no OS or PFS difference was reported between the two groups, while overall response rate (ORR) was higher with the addition of cetuximab to CT compared to CT alone [19].

Similar ORR results were seen in the OPUS trial. In KRAS wild-type tumors, the addition of cetuximab to FOLFOX-4 was associated with a clinically significant increased chance of response and a lower risk of disease progression. The same results were not seen in the overall population, confirming the relevance of KRAS mutational status [12].

Although the addition of cetuximab to oxaliplatin-containing CT had little survival impact, the CRYSTAL trial showed different results when combining cetuximab to FOLFIRI. A borderline significant PFS increase was seen in the combination arm, although with no OS differences. However, when KRAS mutational status was considered, a significant PFS increase was observed favoring cetuximab [20].

Additionally, in the phase III open-label FIRE-3 trial, cetuximab was compared with bevacizumab, both in combination with FOLFIRI. No differences were observed in the primary endpoint of ORR or in PFS, but the median OS was improved in cetuximab arm [21].

Cetuximab was further compared with bevacizumab, both combined with CT (FOLFOX or FOLFIRI), in the CALGB 80405, with no significant differences in ORR, PFS, or OS [22].

2.1.2 Second- and subsequent-line setting

In the 181 trial, the efficacy and safety of adding panitumumab to FOLFIRI was compared with FOLFIRI alone in RAS wild-type mCRC patients who had failed the initial treatment. Addition of panitumumab to the regimen resulted in a significant PFS improvement, of approximately 2 months. Although not significant, a trend towards an OS benefit was seen with the addition of panitumumab [23].

Conversely, the randomized open-label PICOLLO trial reported no benefit with the addition of panitumumab to irinotecan after progression on fluoropyrimidine, with or without oxaliplatin. However, better PFS and more responses were reported in the panitumumab group [24].

In 2004, Saltz et al. and Cunningham et al. evidenced the role of cetuximab in heavily pretreated patients. Saltz et al. reported a median OS of 6.4 months and a median PFS of 1.4 months in 57 patients receiving cetuximab monotherapy after

progression on irinotecan, and a tumor RR of 8.8% [25]. Cunningham et al. included over 300 patients and investigated the role of cetuximab (with or without irinotecan) after progression on irinotecan. A PFS and ORR benefit was observed, with a numeric but not statistically significant difference also observed in OS (8.6 vs. 6.9 months) [26].

Later, the randomized phase II ASPECCT trial compared panitumumab alone with cetuximab alone as third-line treatment for mCRC patients with RAS wild-type (exon 2) tumors. With OS as primary endpoint, panitumumab was given at a dose of 6 mg/Kg every two weeks and cetuximab at a loading dose of 400 mg/m^2, followed by a weekly dose of 250 mg/m^2. No efficacy differences were observed, with a median OS of 10.4 months for panitumumab and 10.0 months for cetuximab [27].

Setting	Study	Treatment	RR□, %	PFS□, months	OS□, months
1st line	PRIME	PAN+FOLFOX4	59*	10.1*	26.0*
		FOLFOX4	46*	7.9*	20.2*
1st line	PEAK	PAN-mFOLFOX6	64	13.0*	41.3
		mFOLFOX6	61	9.5*	28.9
1st line	PLANET-TTD	PAN-FOLFOX4	74	12.8	39.0
		PAN-FOLFIRI	67	14.8	45.8
1st line	314	PAN-FOLFIRI	RASwt: 56* RASmt: 38*	RASwt: 8.9* RASmt: 7.2*	NR
1st line	COIN	CET-OXAL	64*	8.6	17.9
		OXAL	57*	8.6	17.0
1st line	OPUS	CET-FOLFOX4	61*	8.3*	22.8
		FOLFOX4	37*	7.2*	18.5
1st line	CRYSTAL	CET-FOLFIRI	46.9*	9.9*	24.9
		FOLFIRI	38.7*	8.7*	21.0
1st line	FIRE-3	CET-FOLFIRI	62.0	10.0	28.7*
		BEVA-FOLFIRI	58.0	10.3	25.0*
1st line	CALGB 80405	CET-FOLFOX/FOLFIRI	59.6	10.5	30.0
		BEVA-FOLFOX/ FOLFIRI	55.2	10.6	29.0
2nd or greater	181	PAN-FOLFIRI	36*	5.9*	14.5
		FOLFIRI	10*	3.9*	12.5
2nd or greater	PICOLLO	PAN- CPT-11	34*	HR 0.78*	10.4
		CPT-11	12*		10.9
2nd or greater	Saltz, 2004	CET	8.8	1.4	6.4
2nd or greater	Cunningham, 2014	CET + CPT-11	22.9*	4.1*	8.6
		CET	10.8*	1.5*	6.9
2nd or greater	ASPECCT	PAN	22.5	4.1	10.4
		CET	20	4.4	10.0

BEVA, bevacizumab; CET, cetuximab; CPT-11, irinotecan; mt, mutated; NR, not reported; ORR, overall response rate; OS, overall survival; OXAL, oxaliplatin-containing chemotherapy regimen; PAN, panitumumab; PFS, progression-free survival; wt, wild-type.
□Results for the KRAS wild-type subgroup, except if clearly stated.
**Difference between groups is statistically significant ($p < 0.05$).*

Table 1.
Targeted therapies against EGFR in colorectal cancer.

2.1.3 Maintenance/treatment intensification

Regarding maintenance and treatment intensification, three clinical trials are worth mentioning: VOLFI, VALENTINO, and SAPPHIRE.

VOLFI was a randomized open-label phase II trial comparing the addition of panitumumab to FOLFOXIRI CT regimen. An ORR of 87,3% was seen in the FOLFOXIRI plus panitumumab arm, which was higher compared with FOLFOXIRI alone. PFS was similar in both arms, whereas OS showed a trend in favor of panitumumab [28]. This was the highest ORR reported in mCRC, suggesting that these protocols can be considered to obtain maximum cytoreduction in selected patients.

The VALENTINO trial, an open-label phase II trial, investigated maintenance therapy with panitumumab (induction therapy with FOLFOX-4 + panitumumab followed by maintenance with panitumumab ±5FU/LV). The study hypothesis that panitumumab alone was not inferior to the combination as maintenance therapy could not be proven. ORR and OS results did not differ between the two arms [29].

In the SAPPHIRE trial, patients received six cycles of mFOLFOX6 plus panitumumab as induction therapy. Patients who completed induction therapy without progression were then randomized to mFOLFOX6 plus panitumumab (group A) or 5-FU/LV plus panitumumab (group B). PFS, RR, OS, and time to treatment failure were similar between groups, adding to the concept that planned discontinuation of oxaliplatin after six cycles of mFOLFOX6 is a potential treatment option for mCRC patients, achieving similar efficacy while reducing oxaliplatin-associated peripheral neuropathy compared with mFOLFOX6 plus panitumumab [30].

2.2 Resistance mechanisms

Although anti-EGFR therapy has shown benefit in a particular subgroup of CRC patients, primary or innate resistance is high among unselected patients. Furthermore, even patients that initially respond to cetuximab and panitumumab, eventually develop resistance and relapse under these therapies (secondary resistance). Knowledge of the resistance mechanisms associated with the EGFR pathway is crucial to improve therapy efficacy.

2.2.1 RAS-RAF *mutations*

RAS–RAF-MAPK is an EGFR direct downstream signaling pathway, highly deregulated in CRC. Mutations frequently found in these family members generally lead to protein constitutive activation independently of the upstream signaling cascade. Over the last decade, analysis of retrospective clinical trial data (in particular of the OPUS, CRISTAL, and PRIME trials) led to the discovery that patients harboring *RAS* (*KRAS* and *NRAS*) and *BRAF* (specially V600E) activating mutations do not benefit from cetuximab and panitumumab treatment, and that it could even be detrimental for them [31]. These results have led the European Medicines Agency (EMA) and Food and Drug Administration (FDA) to recommend against the use of EGFR-targeted therapies in patients harboring *RAS* and *BRAF* mutations. These mutations are currently the only clinically validated predictive marker of resistance to anti-EGFR therapies in CRC.

2.2.2 PIK3CA *gene and PTEN expression*

Although *RAS* and *RAF* mutations are effective in predicting resistance, not all wild-type patients respond to cetuximab and panitumumab. The EGFR receptor also signals through the PI3K-AKT pathway, resulting in tumor cell proliferation

and survival [32]. Retrospective studies of cetuximab treatment in chemorefractory metastatic CRC patients revealed that *KRAS* wild-type patients with *PIK3CA* mutations in exon 20 (but not in exon 9) have lower response rates compared to unmutated patients (0.0% vs. 36.8%; 95% confidence interval [CI] 0.00–0.89; p = 0.029) [33]. PTEN is another potential marker of response to anti-EGFR therapy, given its inhibitory role on PI3K-AKT signaling pathway. Although PTEN studies are scarce and inconclusive, some works suggest that loss of PTEN expression (measured by immunohistochemistry [IHC]) is associated with decreased RR, PFS, and OS in metastatic CRC patients treated with anti-EGFR therapy [34, 35].

2.2.3 Other resistance pathways

Evidence from cellular studies has suggested that constitutive activation of other EGFR downstream pathways, such as those including the JAK–STAT family, are implicated in resistance to the anti-EGFR gefitinib [36, 37].

Additionally, amplification of other receptor tyrosine kinases (RTKs) has been proposed as a resistance mechanism to anti-EGFR therapies. Expression of VEGF-1 or its receptor (VEGFR) has been associated with cetuximab resistance in both preclinical models and metastatic CRC patients [38]. Bertotti et al. reported that human epidermal growth factor receptor 2 (HER2) gene amplification correlated with cetuximab resistance in a patient-derived xenograft mouse model [39]. Besides HER2, also HER3 has been described to have a role in resistance mechanism to EGFR-targeted therapies. In a cohort of metastatic CRC patients treated with irinotecan and cetuximab, HER3 overexpression was associated with lower PFS and OS [40].

Finally, growing evidence implicates the MET pathway in both primary and secondary resistance mechanisms to mAbs in *KRAS* wild-type patients, through MET amplification or hepatocyte growth factor (HGF) increased expression [41]. In a randomized phase II clinical trial of chemorefractory *KRAS* wild-type anti-EGFR-naïve patients, the combination of anti-HGF mAbs and panitumumab led to higher RR and a trend towards better outcomes in the population with MET overexpression [42].

2.3 BRAF

Although RAS mutations are negative predictors of efficacy in cetuximab and panitumumab treatment, it is acknowledged that not all RAS wild-type patients respond to these agents. To investigate this, research efforts were driven downwards in the MAPK pathway, putting the spotlight on BRAF. This is the main effector in EGFR pathway and is usually mutated in 5–10% of mCRC patients. BRAF and KRAS are usually mutually exclusive, with BRAF V600E mutation (class I) accounting for most alterations found and conferring worse prognosis to these patients.

Regardless of EGFR blockade, BRAF mutations can keep the downstream signaling persistently activated, suggesting that they can confer EGFR blockade resistance. In fact, in a retrospective trial, De Roock et al. showed that chemorefractory mCRC patients with *BRAF* V600E mutations have significantly lower RR to cetuximab than patients with wild-type tumors (8.3% vs. 38.0%; odds ratio 0.15; p = 0.0012) [43]. Several multicentre trials and meta-analyses have subsequently confirmed that *BRAF* V600E mutation results in shorter PFS and OS compared to the wild-type phenotype, emphasizing its role in resistance to anti-EGFRs in patients with chemorefractory mCRC.

Multiple combinations with drugs targeting the MAPK pathway have been tested in BRAF-mutant CRC. Monotherapy results were disappointing when compared to the clinical activity seen in melanoma. In contrast to melanoma, CRC expresses high levels of activated EGFR, which reactivate the MAPK pathway after

single BRAF inhibition [44, 45]. In view of the possibility of therapy resistance via EGFR signaling feedback activation, the trial was amended to include safety and efficacy assessment of vemurafenib combined with cetuximab in a heavily pretreated population, with positive results (median PFS of 3.7 months and median OS of 7.1 months). Similar results were observed when combining dabrafenib with panitumumab (median PFS of 3.5 months) and encorafenib with cetuximab (RR of 23.1%, median PFS of 3.7 months), with phase II results of the latter showing a median PFS of 4.2 months and an ORR of 22% [46].

CT was also combined with BRAF and EGFR inhibition in a phase II trial of irinotecan, cetuximab, and vemurafenib. A total of 106 patients were enrolled, with the study reporting a PFS benefit of 4.3 months with the addition of vemurafenib compared to 2.0 months in the control arm [47].

BRAF inhibition can also induce EGFR overactivation or PI3K modulation, and triplet combos targeting EGFR, MAPK, and PI3K have shown positive results. The MEK116833 trial included 24 patients receiving full-dose combination of panitumumab, trametinib, and dabrafenib and reported an ORR of 21%, a median PFS of 4.1 months, and an OS of 9.1 months. Additionally, a randomized phase II trial combining encorafenib, cetuximab, and the PI3K inhibitor alpelisib reported a median PFS of 5.4 months and an ORR of 27% in interim analysis [48–51].

More recently, the phase 3 BEACON trial investigated the doublet of encorafenib plus cetuximab and the triplet of encorafenib plus cetuximab plus binimetinib in patients with *BRAF*-mutant CRC after one or two prior regimens. The updated analysis confirmed an ORR of 27% with the triplet versus 20% with the doublet versus 2% in the control arm. Median OS was 9.3 months with the duplet and 5.9 months in the control group (hazard ratio [HR] 0.61). The benefit was seen across all subgroups. Numerically identical median OS was observed when comparing the triplet and doublet, with higher toxicity for the triplet (mainly gastrointestinal toxicity and anemia). Subgroup analysis suggested survival benefits in some subgroups, such as those with ECOG 1, three or more organs affected, and higher levels of C-reactive protein and with unresected primary tumors, suggesting that patients with higher disease burden and inflammatory drive could benefit from triple therapy. PFS was also comparable between doublet and triplet and clearly superior to the control arm [52, 53].

2.4 HER2-amplified CRC

HER2 is a growth factor receptor involved in CRC development and progression. HER2 amplification is relatively uncommon, reported in only 3–5% of metastatic CRC patients with wild-type KRAS and wild-type BRAF [54].

Trastuzumab is a monoclonal antibody targeting HER2. The phase II HERACLES trial included mCRC patients with KRAS wild-type, HER2-positive (defined as 2+/ 3+ HER2 score in >50% of cells by IHC or HER2:CEP17 ratio > 2 in >50% of cells by fluorescent *in situ* hybridization [FISH]) tumors who were refractory to standard therapy with EGFR inhibitors and were treated with trastuzumab and lapatinib. ORR was 30%, with one complete response, and median OS was 46 weeks [55]. The most common AEs were diarrhea, rash, and fatigue (78%, 48%, and 48%, respectively). These findings suggested that HER2 positivity was an important driver in CRC. In the phase IIa multi-basket MYPATHWAY trial, patients with HER2-amplified tumors (including CRC) received dual blockade therapy with pertuzumab and trastuzumab. Preliminary results showed promising response, with an ORR of 37.5%, and suggested durable responses with HER2-targeting agents, with a median DoR of 11 months [56].

Both the TRIUMPH (trastuzumab and pertuzumab) and MOUNTAINEER (trastuzumab and tucatinib) trials reported high response rates (35% and 52%,

respectively) and encouraging median PFS (4.0 and 8.1 months, respectively), supporting dual HER2 blockade in patients with HER2-amplified metastatic CRC [57, 58]. Conversely, the combination of pertuzumab and TDM-1 did not show an enhanced objective response in the HERACLES-B trial, although achieving a similar disease control to the HERACLES-A trial (ORR of 10% and median PFS of 4.8 months at cut-off) [59].

Regarding new antibody-drug conjugates, the phase 2 DESTINY-CRC01 trial, of trastuzumab deruxtecan (T-DXd; DS-8201) and also in patients with metastatic HER2-amplified CRC, reported significant responses (ORR of 45.3%, disease control rate [DCR] of 83%), including in patients previously submitted to HER2 blockade [60].

3. VEGF pathway

Tumor angiogenesis is one of the hallmarks of cancer and a key process in tumor development [61, 62]. One of the most relevant pathways involved in angiogenesis is the vascular endothelial growth factor/vascular endothelial growth factor receptor (VEGF/VEGFR) signaling pathway. VEGF-A is a heparin-binding glycoprotein with potent angiogenic activity. VEGF is produced by different cell types, such as immune cells, fibroblasts, and cancer cells, in response to tumor hypoxia via hypoxia-inducible factor (HIF)-1a pathway, inducing an angiogenic switch [63]. Overproduction of pro-angiogenic growth factors leads to formation of chaotic blood vessels in the tumor, with a leaky endothelial wall [64].

3.1 VEGF inhibition in mCRC

In CRC, primary tumor growth and distant metastases development are highly dependent on new vessel formation, making VEGF signaling pathway an attractive therapeutic target. Inhibition of VEGF signaling pathway can be achieved through neutralizing antibodies binding VEGF ligands or blocking VEGFR, or tyrosine kinase inhibitors (TKIs) blocking intracellular VEGFR-dependent signaling [65].

Bevacizumab. The first angiogenesis inhibitor approved for mCRC was bevacizumab, an immunoglobulin G (IgG)1 monoclonal antibody with affinity to VEGF-A. Several trials have evaluated the benefit of adding bevacizumab to cytotoxic regimens as first-line treatment of patients with mCRC, with inconsistent PFS and OS results (**Table 2**).

A phase III trial conducted by Hurwitz et al. compared the efficacy of irinotecan, bolus fluorouracil, and leucovorin (IFL) plus bevacizumab versus IFL plus placebo in untreated mCRC patients. Bevacizumab was intravenously administered at a dose of 5 mg/kg every two weeks along with CT. Bevacizumab arm showed a meaningful improvement in OS (20.3 versus 15.6 months in placebo arm) and PFS (10.6 versus 6.2 months in placebo arm) [66]. Saltz et al. assigned mCRC patients in a 2x2 factorial design to receive CAPOX or FOLFOX4 followed by bevacizumab or placebo as first-line treatment. Median PFS was higher in the bevacizumab group compared with placebo (9.4 versus 8.0 months). OS differences did not reach statistical significance, but only 29% of bevacizumab recipients were treated until disease progression or toxicity [67]. For elderly patients with untreated and unresectable mCRC not candidates for oxaliplatin- or irinotecan-based therapies, the phase III AVEX trial compared the efficacy and safety of capecitabine combined with bevacizumab versus capecitabine alone. Capecitabine was given at a dose of 1000 mg/m^2 orally twice a day on days 1–14 and bevacizumab was administered intravenously at a dose of 7.5 mg/kg on day 1, every 21 days. Longer PFS was

documented in the bevacizumab arm (9.1 versus 5.1 months for capecitabine alone), with acceptable tolerance. Grade ≥ 3 adverse events reported in the combination arm included hand-foot syndrome (16%), diarrhea (7%), and venous thromboembolic events (8%) [68].

Despite these results, the 2015 phase III ITACa trial reported no statistically significant PFS and OS differences when bevacizumab was added to standard first-line CT (FOLFIRI or FOLFOX4) [69]. Other previous trials reported the same negative results. Considering these discrepancies, a 2017 meta-analysis based on 9 studies examined the survival impact of bevacizumab plus CT in first-line treatment of mCRC patients, showing that the combination significantly prolonged PFS (HR 0.66; $p < 0.0001$) and OS (HR 0.84; $p = 0.0001$) compared with CT alone. Subgroup analyses suggested that irinotecan-based regimens might be a better partner for bevacizumab than oxaliplatin-based regimens, with superior PFS and OS benefit [70].

Sidedness of the primary tumor is known to be an important prognostic factor in metastatic setting of CRC, with worst survival outcomes for right-sided tumors. Several clinical trials investigated the prognostic role of bevacizumab in the treatment of patients with right-sided and left-sided CRC. A post-hoc analysis of 16 randomized trials including PEAK, FIRE-3, and CALGB/SWOG trials showed that right-sided tumors have impaired CT sensitivity, while addition of bevacizumab to cytotoxic regimens can be an optimal first-line treatment for RAS-wild-type right-sided mCRC [71].

Although continuing bevacizumab with second-line chemotherapy showed benefit after disease progression, other anti-VEGF drugs should be considered for fast progressors (PFS <3–4 months) [72].

In patients with unresectable mCRC who are not candidates for intensive therapy, the ongoing phase III SOLSTICE trial is currently comparing trifluridine/tipiracil (TAS-102) plus bevacizumab versus capecitabine plus bevacizumab as first-line treatment [73].

Aflibercept. Aflibercept is a recombinant fusion protein composed by VEGF-binding portions from VEGFR-1 and -2 extracellular domains fused to the Fc portion of human IgG1. It acts by blocking the activity of VEGF-A and -B, preventing their binding to VEGFR on endothelial and tumor cells [74].

The role of aflibercept was evaluated in the phase III VELOUR trial, of mCRC patients previously treated with oxaliplatin-based regimens in first line, including with bevacizumab. Second-line FOLFIRI was intravenously administered with placebo or aflibercept at the dose of 4 mg/kg every two weeks. Aflibercept improved the median OS (13.50 vs. 12.06 months) and median PFS (6.90 versus 4.67 months) compared to placebo [74]. These results lead to approval of the drug in combination with FOLFIRI as second-line treatment for patients pretreated with oxaliplatin-based doublet with bevacizumab. The most common grade ≥ 3 AEs reported in the VELOUR trial included neutropenia, diarrhea, stomatitis, hypertension, and fatigue. Additionally, there was no evidence of greater toxicity in patients previously treated with bevacizumab [74].

More recently, the phase II AFFIRM trial investigated the addition of aflibercept to first-line oxaliplatin-based regimens in mCRC patients. Patients received mFOLFOX6 plus aflibercept or mFOLFOX6 alone. Despite VELOUR results, this study did not reach the primary endpoint of PFS. Adding aflibercept to first-line mFOLFOX6 did not increase efficacy and was associated with higher toxicity [75].

Ramucirumab. Ramucirumab is a human IgG1 monoclonal antibody against VEGFR-2. Efficacy and safety of ramucirumab in combination with second-line FOLFIRI was evaluated in the phase III RAISE trial. Patients with progressive mCRC during or after first-line treatment with bevacizumab, oxaliplatin, and

fluoropyrimidine were randomized to receive intravenous ramucirumab 8 mg/kg plus FOLFIRI or placebo plus FOLFIRI every 2 weeks. Ramucirumab significantly improved survival in this subpopulation, reaching a median OS of 13.3 months, against 11.7 months in the placebo arm. Grade ≥ 3 AEs included neutropenia (38%), hypertension (11%), diarrhea (11%), and fatigue (12%). Febrile neutropenia was only reported in 3% of patients and most toxicities reported were manageable [76]. This trial lead to the approval of ramucirumab in combination with FOLFIRI in the second-line setting of mCRC previously treated with bevacizumab, oxaliplatin, and fluoropyrimidine in first line.

Regorafenib. The only TKI approved for mCRC treatment is regorafenib, a multi-kinase inhibitor of angiogenic pathway members, including VEGFR-1 and -2, platelet-derived growth factor receptor (PDGFR)-β, and tyrosine kinase with immunoglobulin-like and EGF-like domains 2 (TIE2) [77].

Several phase III trials evaluated the role and efficacy of regorafenib as single-agent in mCRC patients progressing after several standard lines of treatment (**Table 2**). The CORRECT trial was the first to compare treatment with regorafenib 160 mg daily for 21 days, every 28-day cycle, versus placebo. Final study results reported a quality of life (QoL) and OS (6.4 vs. 5.0 months in placebo arm) improvement in favor of regorafenib [78]. The phase III CONCOUR trial was similar to the CORRECT trial but exclusively recruited Asian patients, holding similar OS results [79]. The CONSIGN trial was designed to specifically evaluate regorafenib safety. In a total of 2864 patients (median age of 62 years), the most common grade ≥ 3 AEs were hypertension (15%), hand-foot syndrome (14%), fatigue (13%) and diarrhea (5%). Grade ≥ 3 laboratory toxicities included elevated alanine aminotransferase (6%), aspartate aminotransferase (7%), and bilirubin (13%) [80].

3.2 Resistance to anti-VEGF drugs

Despite the outcome benefits seen with anti-VEGF agents in CRC, these are usually transient and followed by relapse and tumor growth [81]. Several resistance mechanisms to anti-VEGF therapies have been described, including VEGF axis-dependent alterations, non-VEGF axis-dependent upregulation, and stromal cell interactions [82].

3.2.1 VEGF-dependent pathways

Upregulation of alternative VEGFR-2 angiogenic ligands, such as VEGF-C, −D, and placental growth factor (PIGF), can bypass VEGF-A inhibition and elicit bevacizumab resistance [82]. In a phase II trial, Kopetz et al. showed that PlGF, VEGF-C, and VEGF-D plasma levels in mCRC patients receiving FOLFIRI plus bevacizumab were elevated prior to and at the time of disease progression [83].

3.2.2 Non-VEGF-dependent pathways

Complementary angiogenic pathways other than VEGF/VEGFR signaling exert control on tumor angiogenesis and may explain acquired resistance to anti-VEGF therapies. These pathways involve members of the platelet-derived growth factor (PDGF) family, HIF, members of the fibroblast growth factor (FGF) family, angiopoietin (Ang), and Notch [84, 85].

The PDGF family consists of five ligands that bind to tyrosine kinases PDGFR-α and -β, activating downstream signal transduction pathways, as PI3K/Akt and PLCγ. PDGF-C was shown to be upregulated in cancer-associated fibroblasts (CAFs) of anti-VEGF-resistant tumors in vivo [86], making it a possible resistance mediator.

Study	Treatment	PFS, months	OS, months	HR (p-value)
Hurwitz et al. (III)	BEVA-ILF PLACEBO-IFL	10.6* 6.2*	20.3 15.6	PFS - 0.54 (<0.001) OS - 0.66 (>0.001)
Saltz et al. (III)	XELOX BEVA-FOLFOX PLACEBO	9.4* 8.0* -	23.3 19.9 -	PFS - 0.83 (0.002) OS - 0.89 (0.077)
AVEX (III)	BEVA-CAP CAP	9.1* 5.1*	—	PFS - 0.53 (<0.001)
ITACa (III)	BEVA-FOLFIRI/FOLFOX PLACEBO-FOLFIRI/FOLFOX	9.6 8.4	—	PFS - 0.86 (0.182)
SOLSTICE (III)	BEVA-Trifluridine/tipiracil BEVA-CAP	—	—	Ongoing
VELOUR (III)	Aflibercept-FOLFIRI PLACEBO-FOLFIRI	6.90* 4.67*	13.50* 12.06*	PFS - 0.758 (<0.001) OS - 0.817 (0.003)
AFFIRM (II)	Aflibercept-FOLFOX PLACEBO-FOLFOX	8.48 8.77	—	PFS - 1.00
RAISE (III)	Ramucirumab-FOLFIRI PLACEBO-FOLFIRI	—	13.3* 11.7*	OS - 0.844 (0.022)
CORRECT (III)	Regorafenib PLACEBO	—	6.4* 5.0*	OS - 0.77 (0.005)
CONCOUR (III)	Regorafenib PLACEBO	—	8.8* 6.3*	OS - 0.55 (<0.001)
CONSIGN (III)	Regorafenib	AEs: hypertension (15%), hand-foot skin reaction (14%), fatigue (13%), diarrhea (5%), and elevated aminotransferase (6%), aspartate aminotransferase (7%), and bilirubin (13%).		

AEs, adverse events; BEVA, bevacizumab; CAP, capecitabine; HR, hazard ratio; OS, overall survival; PFS, progression-free survival.
**Difference between groups is statistically significant ($p < 0.05$).*

Table 2.
Targeted therapies against VEGF in colorectal cancer.

HIF-1 is a transcription factor with a key role in cellular response to reduced oxygen levels. Among its multiple downstream effects is induction of VEGF-A, VEGFR, PIGF, and PDGF expression [85].

Growth factors of the FGF family are potent mediators of tumor angiogenesis. Binding of FGF to fibroblast growth factor receptor (FGFR) tyrosine kinase activates downstream pathways such as MAPK/ERK, PI3K/Akt, and STAT [86], acting synergistically with VEGFA to induce angiogenesis via endothelial cell proliferation, survival, and migration [87]. FGF-2 upregulation is observed in anti-VEGF-resistant tumors, especially in tumors exposed to a hypoxic environment, [86] while FGF-2 blockade results in decreased tumor growth in *in vivo* models [88].

Ang-Tie signaling is a vascular-specific pathway essential for blood vessel development and vascular permeability regulation. Ang-2 acts as an antagonist of the Tie2 receptor, leading to development of vascular sprouts in the context of VEGF exposure [86]. mCRC patients with poor bevacizumab response showed high serum Ang2 levels, suggesting its relevance in resistance to anti-angiogenic therapy [89].

Delta-like ligand 4 (DII4) is a Notch ligand overexpressed in several solid malignancies, including CRC. DII4 upregulation is thought to contribute to

bevacizumab resistance, which can be overcome by Notch inhibition with a γ-secretase inhibitor [90].

TGF-β is a ligand for type II TGF-β receptors and endoglin (CD105). It has important regulatory functions in angiogenesis, either directly, or indirectly by activating fibroblasts to produce extracellular matrix and stimulating the tube formation in endothelial cells [91]. Anti-VEGF therapy-resistant tumors can exhibit high levels of TGF-β1 expression. Additionally, in preclinical models VEGF pathway blockade led to increased CD105 levels, suggesting a role for CD105 in anti-VEGF therapy resistance [92].

3.2.3 Stromal cell interactions

It has been recently suggested that tumor stromal cells and bone marrow-derived cells (BMDCs) recruited to the tumor microenvironment by secreted cytokines play an important role in acquired resistance to anti-VEGF therapies [81].

CAFs entail a large portion of stromal cells present in the tumor environment. These cells secrete a number of pro-angiogenic mediators, including IGF, FGF, EGF, cytokines, and chemokines, and are capable of recruiting endothelial progenitor cells (EPCs) to the tumor site [93, 94]. Interestingly, Kinugasa et al. showed that CAFs from anti-VEGF-resistant tumors express high levels of CD44, a marker for cancer stem cells and cytotoxic resistance. CAFs can hence be considered a promising target for overcoming resistance to anti-angiogenic agents [95].

BMDCs are comprised of endothelial and pericyte progenitors, macrophages, and myeloid-derived suppressor cells (MDSCs) [96]. Preclinical models suggest that EPCs in the tumor microenvironment are able to secrete different proangiogenic factors and accelerate angiogenesis [97]. More importantly, endothelial precursor cells can differentiate into endothelial cells and participate in new vessel formation [98, 99].

Tumor-associated macrophages (TAMs) are also involved in angiogenesis. VEGF blockade by bevacizumab seems to promote TAM proliferation and reprogramming to pro-angiogenic macrophages [81]. This type of macrophages can secrete VEGF-A, TNFα, and IL-8, all of which affect different stages of angiogenesis by modifying the local extracellular matrix, promoting proliferation and migration of endothelial cells, and inhibiting development of differentiated capillaries [81].

A study by Shojaei et al. demonstrated that MDSCs were present in higher levels in anti-VEGF-resistant tumors and were functionally different from those in anti-VEGF-sensitive tumors. This population was able to sustain tumor growth even in presence of anti-VEGF inhibitors, although the exact mechanism behind this is not been fully established [100].

CD4+ T-helper cells mediate anti-VEGF resistance through IL-17 production in the tumor microenvironment and BMDC recruitment. These cells have been shown to regulate secretion of several proangiogenic factors from CAFs and other stromal cells. Additionally, Numasaki et al. reported that tumor microvessel density correlates with levels of infiltrating IL-17-producing CD4 T-cells [25, 42, 81, 101].

3.3 Anti-EGFR and -VEGF safety profile

The main side effects of the anti-EGFR therapies cetuximab and panitumumab are dermatological toxicities, reported in 85–96% of patients (**Table 3**) [102]. The most common AE is papulopustular skin rash, generally developing over a period of 6 weeks after starting treatment and potentially impacting quality of life and therapy adherence. General prevention and management principles include the use of skin moisturizer, sunscreen, hydrocortisone cream, and oral tetracycline. The STEPP trial compared pre-emptive with reactive skin treatment and showed an

over 50% reduction in grade ≥ 2 skin toxicities and less QoL impairment with the pre-emptive compared with reactive treatment [103]. In cases of grade 3 rash, treatment should be delayed until toxicity has resolved to grade 2 or less and dose should be reduced in a second occurrence. In grade 1 or 2 rash, dose reduction is not indicated. Other dermatological symptoms, including hair growth, periungual and nail plate abnormalities, xerosis, telangiectasias, and pruritus can occur at lower rates [102].

Infusion reactions commonly occur with cetuximab and should be prevented with premedication, antihistamines, and corticosteroids. Other adverse effects, like hypomagnesemia, ocular toxicities as conjunctivitis and blepharitis, and less commonly diarrhea, can also occur [104]. Toxicity management is grade-depend and, in some cases, should be addressed by a multidisciplinary team.

The main anti-VEGF side effects are cardiovascular and kidney problems (**Table 3**). Hypertension has been observed at high rates in all phase III studies of anti-VEGF drugs and is normally manageable with standard antihypertensive medications, but this treatment should not be initiated in patients with uncontrolled hypertension. Proteinuria is another side effect, defined as protein content in the urine >300 mg/dL. No standard treatment is established, but anti-angiogenic drugs should be disused if protein content in the urine is >2 g/24 h, and evaluation by a nephrologist should be considered. Hand-foot syndrome is also common with this class of drugs [105].

Bevacizumab has also been associated with other side effects, like thromboembolic events (8%), delayed wound healing, bleeding, fistulae, and gastrointestinal

Target	Effect	Drug-incidence	Prevention/treatment	Dose reduction/delay treatment
EGFR	Rash	C 52–89% P 20–50%	Skin moisturizer, sunscreen, hydrocortisone cream, and oral tetracycline	Reduction in 2nd G3 occurrence, delay until ≤ G2
	Infusion reactions	C 14–21% P-3%	Antihistamines and corticosteroids Low rate, gradual titration	Grade dependent
	Hypomagnesemia	C 4–38% P 27%	Magnesium replacement	Some G3/4 toxicity delay until recovery
	Diarrhea	2% G3/4	Loperamide, hydration, electrolyte replacement, hospitalization	Reduction in 1st G3 or 2nd G2 occurrence
VEGF	Hypertension	B 25% A 42.4% Reg 15% Ram 11%	Blood pressure monitoring, antihypertensive drugs	Cease if G4 or persisting G3 toxicity
	Proteinuria	18.7%	Screening for proteinuria angiotensin receptor blockers	Discontinue if nephrotic syndrome
	Hand-foot syndrome	B 16% Reg 14%	Emollient, analgesia	Reduction in 1st G3 or 2nd G2 occurrence, delay until ≤ G1
	Thromboembolic events	B 8%	Anticoagulation therapy	Cease bevacizumab

A, aflibercept; B bevacizumab; C cetuximab; EGFR, epidermal growth factor receptor; G grade; P, panitumumab; Ram, ramucirumab; Reg, regorafenib, VEGF, vascular endothelial growth factor.

Table 3.
Adverse effects of any severity with anti-EGFR and -VEGF therapies.

perforation (1.7%). Bevacizumab treatment should be ceased in cases of hemorrhagic events ≥grade 3, pulmonary embolism, cerebrovascular events or arterial insufficiency, arterial thromboembolic events, grade 4 or persistent grade 3 hypertension, nephrotic syndrome, or gastrointestinal perforation [106]. Potentially life-threatening events have occurred only in a small number of patients, with bevacizumab being well tolerated by the majority.

4. Other targets

4.1 NTRK fusions

The constitutive activation of RTKs promoted by genomic translocations play an important role in tumorigenesis across different malignancies, including CRC. Examples include ALK, ROS1, and NTRK1–2-3 (NTRK), which altogether occur in 0.2–2.4% of CRCs and may represent new therapeutic targets (**Table 4**) [107].

The NTRK (neurotrophic tropomyosin receptor kinase) 1, 2, and 3 genes encode three tropomyosin receptor kinase (TRK) receptors —TrkA, TrkB, and TrkC— which are transmembrane proteins [2, 108, 109]. Gene fusions involving those genes lead to constitutively activated NTRK proteins and, consequently, tumorigenesis [107]. The prevalence of NTRK fusions in mCRC is estimated to be 0.5–2.0% [110], but increases to 4% in microsatellite instability-high (MSI-H) mCRC [2].

NTRK gene rearrangements are more commonly detected in non-Lynch syndrome MSI-H/ deficient mismatch repair (dMMR) tumors with MLH1 promoter hypermethylation and wild-type BRAF/KRAS/NRAS, and define a molecular subgroup associated with poor prognosis [111]. They are also more frequent in elderly females with right-sided tumors [107, 109, 112].

Fusion-detection options include targeted DNA and RNA panels, RNA sequencing, FISH, and IHQ [2]. Recent ESMO recommendations for NTRK fusion detection state that, in tumors with low NTRK fusion frequency, as mCRC, detection can be done via one-step next-generation sequencing (NGS) or via IHQ followed by NGS (if IHQ positive) [113].

Larotrectinib and entrectinib are TRK inhibitors approved by the FDA and EMA in more than 10 tumor types. Larotrectinib, a small-molecule inhibitor targeting all three TRK proteins, has been tested in the multicenter single-arm LOXO-TRK-14001, SCOUT, and NAVIGATE clinical trials [111]. Larotrectinib at the dose of 100 mg twice daily showed a good safety profile and good responses (75% of ORR, 1-year PFS of 55%) [114]. In November 2018, the FDA granted accelerated tissue-agnostic approval to larotrectinib for solid tumors with NTRK gene fusions [2, 111, 112] Entrectinib is an oral pan-TRK, -ROS1, and -ALK inhibitor that is clinically active in patients with NTRK-rearranged tumors and is able to penetrate the blood–brain barrier [107]. Three clinical trials (ALKA-372-001, STARTRK-1, and STARTRK-2) have investigated this agent [107]. Pooled analyses of the three trials presented at the ESMO 2018 Congress and ASCO 2019 Meeting showed that entrectinib induced clinically meaningful durable responses in patients with solid tumors with or without metastatic central nervous systemic disease harboring NTRK fusions [111].

The second-generation TRK inhibitor BAY2731954 (formerly known as Loxo-195) and the next-generation ROS1, pan-TRK, and ALK inhibitor repotrectinib are being tested, with promising results [111].

As already shown with BRAF V600E mutations, patients with ALK-, ROS-, and NTRK-rearranged tumors seem to derive no benefit from treatment with anti-

EGFR monoclonal antibodies [107]. Additionally, the high prevalence of MSI-H status in rearranged tumors opens the way for evaluation of new combination approaches including targeted (ALK, ROS1, TrkA-B-C) and immunotherapy agents [107].

Regarding resistance mechanisms, a dose-dependent effect seems to affect mutation emergence. Two mutations have been associated with entrectinib resistance: NTRK1 p. G667C and NTRK1 p.G595R [108]. For larotrectinib, three different mutational categories have been described: solvent front mutations (NTRK1 p. G595R, NTRK3 p.G623R); gatekeeper mutations (NTRK1 p.F589L); and xDFG mutations (NTRK1 p.G667S, NTRK3 p.G696A). Novel agents under development intend to overcome NTRK1 p.G595R-mediated resistance to TRK inhibitors [115].

4.2 MET alterations

The mesenchymal-epithelial transition (MET) protooncogene (also known as N-methyl-N′-nitroso-guanidine human osteosarcoma transforming gene) encodes for c-MET, a receptor with tyrosine kinase activity targeting HGF. Activation of this pathway has been implicated in CRC metastatic progression [2].

MET receptor tyrosine kinase can be overexpressed in 50–60%, amplified in 10%, and mutated in 5% of CRCs [2]. In a study by Lee et al., c-MET overexpression showed no correlation with primary tumor site, histological type, or molecular aberrations, but correlated with shorter OS and was a predictive biomarker of shorter PFS in bevacizumab-treated patients [3].

EGFR and MET are co-expressed in CRC and MET activation has been implicated in resistance to the anti-EGFR therapy [2, 116]. Inhibition of the HGF/c-Met pathway may improve response to EGFR inhibitors in CRC and combination therapy should be further investigated [116]. This supports the hypothesis that anti-EGFR therapy selects MET-amplified (cetuximab- and panitumumab-resistant) preexisting clones, eventually limiting the efficacy of further anti-EGFR therapies [117].

Multiple clinical trials have evaluated MET inhibition, but several of those conducted in mCRC have been unsuccessful [2]. Treatment strategies targeting HGF and c-Met include HGF antagonists, c-Met and HGF-blocking antibodies, and small-molecule c-Met inhibitors [118].

Although MET genomic aberrations are commonly observed in mCRC, these remain in the research setting [2].

4.3 Other rearrangements

4.3.1 ALK/ROS1 translocations

The EML4-ALK fusion gene is produced by inversion in the short arm of chromosome 2, where anaplastic large-cell lymphoma kinase (ALK) joins echinoderm microtubule-associated protein-like 4 (EML4), resulting in a chimeric protein with constitutive ALK activity. ROS1 is an orphan receptor tyrosine kinase phylogenetically related to ALK [110].

ALK and ROS1 gene rearrangements have not been extensively studied in CRC. Around 0.8–2.5% of patients with mCRC have been reported to have either ALK or ROS1 rearrangements [110]. ALK, ROS1, and NTRK fusions occur more frequently in elderly patients with right-sided, RAS wild-type, MSI-H mCRC, and are associated with shorter OS and poor prognosis [107, 110]. The small patient numbers make it challenging to develop a clinical trial of targeted therapies for this patient population [110]. As no FDA-approved agents targeting these genomic alterations

Target	Frequency	Clinicopathological features	Testing methods	Agent	Mechanism of action	Current status
NTRK genes (NTRK 1, NTRK 2, NTRK 3) fusions	0.5–2.0% in mCRC (4% in MSI-H)	Associated with MSI-H/dMMR; wt BRAF/RAS, elderly females and right sided tumors; associated with poor prognosis; resistance to anti-EGFR monoclonal antibodies	IHC and NGS	Larotrectinib Entrectinib	Small molecule inhibitor targeting TRK proteins	Approved by the FDA and EMA
ALK/ROS1	0.8–2.5%	Associated with MSI-H/dMMR; wt BRAF/RAS, elderly females and right-sided tumors; associated with poor prognosis; resistance to anti-EGFR monoclonal antibodies	FISH, RT-PCR, NGS	Clinical trials; Ceritinib	Small-molecule inhibitor targeting ALK/ROS1	Under investigation
FGFR	3–5%	FGFR3 related with worse prognosis	NGS plus FISH	Regorafenib and newly developing FGFR-specific TKIs	Small-molecule inhibitor targeting FGFR signaling	Under investigation
c-Met overexpression	Overexpressed in 50–60%, amplified in 10% and mutated in 5% of CRCs	Shorter OS, shorter PFS with bevacizumab treatment; poor prognosis; resistance to anti-EGFR monoclonal antibodies	IHC	Clinical trials	Under investigation	Under investigation
RET fusions	0.2%	Worse prognosis, poor treatment response, and reduced OS	IHC and FISH	Vandetanib, cabozantinib	Under investigation	Under investigation

dMMR, deficient mismatch repair; FGFR, fibroblast growth factor receptor; FISH, fluorescent in situ *hybridization; IHC, immunohistochemistry; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability-high; NGS, next-generation sequencing; OS, overall survival; PFS, progression-free survival; RT-PCR, reverse transcription polymerase chain reaction; TKI, tyrosine kinase inhibitor; wt, wild-type.*

Table 4.
Summary of new targeted therapies in mCRC.

exist for CRC patients, basket trials (as the TAPUR trial) may give valuable insights in this setting [112].

4.3.2 RET fusions

RET is a proto-oncogene encoding a transmembrane tyrosine kinase receptor for the glial-derived neurotrophic factor family [110].

RET fusions occur in 0.2% of solid tumors, being very typical in specific tumor types, such as thyroid carcinomas [119]. The effect of RET activation is less clear in CRC, but several studies suggest that it might be associated with worse prognosis, poor treatment response, and reduced OS. Due to rarity of this aberration, clinical trials in CRC are not easy to conduct, with data derived mainly from early trials or case reports [110]. Clinicopathological factors associated with RET fusions include right colon location, older age, RAS and BRAF wild-type status, and MSI-H status [119].

4.3.3 FGFR

Fibroblast growth factor receptors (FGFRs) are a subfamily of RTKs occurring in approximately 3–5% of CRC patients [112]. Initial evidence shows poor outcomes associated with FGFR3 alterations [120]. There is no evidence of clinicopathological characteristics related to these alterations [120].

Regorafenib, a multi-kinase inhibitor also targeting FGFR, is currently approved by the FDA for metastatic CRC patients who progressed on frontline therapies. This agent can be considered in CRC patients with FGFR alterations while novel FGFR inhibitors are not available [121]. Newly developed, more potent FGFR inhibitors are currently being investigated in multiple solid tumors [112].

5. Microsatellite instability and immune checkpoints inhibitors

Microsatellite instability (MSI) is currently a key biomarker in CRC, with diagnostic, prognostic, and therapeutic implications. For these reasons, MSI analysis is becoming increasingly important and testing for deficient mismatch repair (d-MMR)/MSI is recommended, both for hereditary syndrome screening and due to prognostic and treatment implications [122].

Inactivation of a DNA mismatch repair (MMR) gene (MLH1, MSH2, MSH6, or PMS2) by mutation or transcriptional silencing results in deficient function of the MMR system, responsible for excising DNA mismatches introduced by DNA polymerase during cell division. This activity loss translates in an accumulation of DNA replication errors and mismatches in repeated sequences, leading to hypermutated tumors [123]. In most cases, d-MMR and MSI arise due to sporadic somatic hypermethylation of MLH1 and other genes, but they can also result from germline mutations in MMR genes and from Lynch syndrome in approximately 3% of all CRCs [124].

The MMR system can be assessed through different approaches, as IHC, polymerase chain reaction (PCR)-based assays, and more recently NGS. IHC looks at MLH1, MSH2, MSH6, and PMS2 staining in tumor samples to identify the protein expression loss that characterizes d-MMR [125]. PCR amplification requires both tumor and matched normal samples. Five microsatellite loci have been PCR-amplified and analyzed by capillary electrophoresis. Instability at more than one locus was defined as MSI-high (MSI-H), at a single locus as MSI-low (MSI-L), and absence of instability at any locus as microsatellite stable (MSS), proficient MMR

(p-MMR) [126]. NGS detection directly targets certain genes, which are genome sequenced to retrieve information on MSI and MMR and tumor mutational burden (TMB), integrating all information in the same test. NGS requires a smaller sample and is more accurate than PCR. Ethical issues may arise with the use of this technique regarding counseling and consent for additional genetic testing [127]. In CRC, MSI varies according to tumor stage, with higher incidence reported in early stages (20% in stages I-II, 12% in stage III) and lower incidence reported in the metastatic setting (4–5%) [128].

5.1 Immune checkpoint inhibitors

The success of immune checkpoint inhibitors (ICI) in d-MMR over the last years has disclosed a new therapeutic scenario. Endogenous peptides are processed and presented on major histocompatibility complex (MHC) class I molecules on the surface of all cells, being recognized by T cell receptors (TCRs). TCR–MHC signaling pathways are modulated by co-stimulatory or co-inhibitory signals. ICI target co-inhibitory receptors, like cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) on T cells, or their ligands, as programmed cell death ligand 1 (PDL-1), on tumor and various immune cells [129]. ICI are approved in several malignancies. In mCRC, phase I trials reported response to immune checkpoint therapy in a subgroup of patients with MSI-H, d-MMR, or high TMB [130].

Pembrolizumab is a humanized IgG4 antibody and was the first anti-PD-1 to show efficacy in d-MMR mCRC (**Table 5**). In the phase II KEYNOTE-016 trial, patients with d-MMR tumors responded better to pembrolizumab (RR of 40%, 20-week PFS of 78%) than MSS tumors (RR of 0%, 20-week PFS of 11%) [131]. In the updated analysis, an ORR of 52%, 2-year PFS of 59%, and OS of 72% was reported for MSI-H CRC [132]. The phase II KEYNOTE-164 trial confirmed the efficacy of pembrolizumab in second-line setting of MSI-H CRC, with an ORR of 33%, median PFS of 2.3 months, and median OS of 31.4 months [133]. Based on these results, pembrolizumab was approved by the FDA for MSI-H/d-MMR unresectable or metastatic CRC after progression on CT. In the phase III KEYNOTE-177 trial, first-line treatment with pembrolizumab in monotherapy significantly reduced the risk of disease progression or death by 40% (HR 0.60; 95% CI 0.45–0.80; p = 0.0004), with a median PFS of 16.5 months versus 8.2 months with CT in MSI-H CRC. The study is ongoing, and OS data will be presented later this year [134]. This led to FDA approval of pembrolizumab in first-line treatment of unresectable or metastatic MSI-H/dMMR CRC.

Nivolumab, a humanized monoclonal IgG4-based PD-1 antibody, showed activity in MSI-H/d-MMR refractory CRC in the phase II CheckMate-142 trial, with an ORR of 31.1% regardless of tumor PD-L1 expression, 1-year PFS of 50%, and OS of 73% [135]. This trial included a cohort of nivolumab in combination with the CTLA-4 inhibitor ipilimumab, which showed a 55% ORR, 71% PFS, and 85% OS. Both nivolumab and the combination of nivolumab plus ipilimumab were approved by the FDA for CT-refractory MSI-H/dMMR mCRC. The immunotherapy doublet was also evaluated in first line in the CheckMate-142 trial, with 1-year PFS and OS of 77% and 83%, respectively, ORR of 60%, and DCR of 84% [136].

Following these studies, MSI status has become a crucial biomarker to define therapeutic options for patients in the metastatic setting.

Other PD-1/PD-L1 inhibitors are under investigation, like atezolizumab, avelumab, and durvalumab, and new immune checkpoint targets are in phase I trials, such as tumor-overexpressed T cell Ig and mucin domain-containing protein 3 (TIM-3), T cell Ig, and T cell-derived lymphocyte activation gene 3 (LAG-3). [137].

Setting	Study	Treatment	RR	PFS	OS	Approval
CT-refractory MSI-H/d-MMR mCRC	Phase II Keynote 164	Pembrolizumab	33%	2.1 m	31.4 m	FDA (1st line, CT-refractory)
1st line MSI-H/d-MMR mCRC	Phase III Keynote 177	Pembrolizumab	43.8%	16.5 m	NR	
CT-refractory MSI-H/d-MMR mCRC	Phase II CheckMate-142	Nivolumab	31%	50%	73%	FDA (CT-refractory)
	Phase II CheckMate-142	Nivolumab + ipilimumab	55%	71%	85%	
1st line MSI-H/d-MMR mCRC	Phase II CheckMate-142	Nivolumab + ipilimumab	60%	77%	83%	Not approved

CT, chemotherapy; dMMR, deficient mismatch repair, FDA, Food and Drug Administration; mCRC, metastatic colorectal cancer; MSI-H, microsatellite instability-high; NR, not reached; OS, overall survival; PFS, progression-free survival; RR, response rate.

Table 5.
Immune checkpoint inhibitors in mCRC.

5.2 Immunotherapy resistance

Most mCRC patients are MSS/p-MMR and results with ICI have been unsatisfactory, with immune resistance mechanisms not clearly elucidated yet. Several trials have been developed exploring ways to overcome this resistance, including by modulating tumor microenvironment, reducing tumor-specific antigen expression, altering immunosuppressive pathways, and activating other immune checkpoint pathways, immune regulatory cells, and cytokines [138]. Combining immunotherapy with CT, radiotherapy, bispecific antibody therapy, other immune checkpoint modulators, and other targeted agents are among strategies explored. The rationale behind this multimodal approach is the potential synergistic effect of targeting different immune escape pathways, resulting in improved response to ICI and patient outcomes [139].

CT has anti-tumor activity due to the direct cytotoxic effect on cancer cells and to stimulating host immune response, and several clinical trials are ongoing investigating the combination of immunotherapy with CT and targeted agents [140]. Radiotherapy can activate the host immune response by upregulating expression of tumor-specific neoantigens through cell damage and increasing membrane MHC class I expression, and several studies are ongoing in CRC combining radiotherapy with ICI. Another combined strategy is ICI and MEK blockers, considering that MEK blockade seems to increase T cell response via upregulation of PD-L1 expression [141]. Following a phase Ib trial of atezolizumab and the MEK inhibitor cobimetinib in MSS CRC, other trials were conducted, with no significant survival improvement [142]. The CEA CD3 TCB (RG7802, RO6958688) is a novel T-cell bispecific antibody targeting the carcinoembryonic antigen (CEA) on tumor cells and CD3 on T cells, which displays anti-tumor activity, leading to increased intra-tumoral T cell infiltration and activation and PD-1/PD-L1 upregulation. CEA-TCB antibody was tested in phase I trials of MSS CRC plus atezolizumab, showing antitumor activity with acceptable toxicity [143].

5.3 Biomarkers

Considering immune side effects associated with ICI and their variable efficacy, it is important to identify biomarkers that help predict response to ICI and select potentially sensitive patients that can be candidates for these agents.

PD-L1 expression level is an established biomarker in some malignancies, but the relationship between PD-L1 positivity and response has not been proven in CRC [144]. TMB has emerged as a marker of response to immunotherapy in some tumors, suggesting that tumor cells with high mutational burden generate and present more peptide neoantigens on their MHC class I molecules, increasing T cell infiltration [145]. In CRC, dMMR/MSI-H tumors have a high mutational burden, as well as some pMMR/MSS, which may present an ultramutated phenotype as DNA polymerase epsilon (POLE) mutations, found in ~1–2% of pMMR CRC. POLE mutations cause an increased immunogenicity and upregulation of immune checkpoint genes, such as PD-1/PD-L1 and CTLA-4, which result in similar clinical responses to dMMR tumors and may predict response to anti-PD-1 therapy [146]. Some case reports link POLE mutations with efficacy to PD-1 blockade, and phase II studies are ongoing in this setting.

The interaction between tumor and microenvironment led to the development of an immunoscore based on calculation of two lymphocytic populations (CD3/CD45-CD8 or CD8/CD45) in the centre and invasive margins of the tumor, which may predict ICI response [147]. Other lines of investigation are being explored, including the study of factors that indicate cytotoxic T cell activity, such as granzymes, perforins, and IFN-γ levels.

CRC is one of the tumor types for which immunotherapy has been less effective. Better knowledge of the molecular immune mechanisms is required to develop predictive biomarkers and effective therapeutic combination strategies, converting "cold" tumors, immune-desert and immunotherapy-resistant, in "hot" tumors, inflamed, infiltrated by the immune system, and immunotherapy responsive.

6. Conclusions

CRC treatment has changed over the last decades, not only by including different chemotherapy agents and combinations, but mainly because new targeted agents have emerged.

In metastatic setting, anti-EGFR and anti-VEGF drugs are widely used and have shown gains in survival and response rate, an important marker in CRC potentially resectable liver metastases. In contrast, several trials with targeted agents have been conducted in the adjuvant setting, without survival benefit. Immunotherapy emerged as a new treatment option with survival benefit, but at the moment it is only effective in a small portion of patients. Several other agents targeting other pathways are emerging, such as NTRK, c-MET, ALK, ROS1, and FGFR inhibitors, with promising results.

In conclusion, patients with CRC are living longer with targeted treatments, but more information about resistance mechanisms and biomarkers is necessary to extend even more their survival gains.

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Conflict of interest

L. Costa performed consulting activities for Amgen, Novartis and Servier outside the scope of this manuscript. The remaining authors declare no conflicts of interest.

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