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Review

New developments in targeted therapy for metastatic colorectal cancer

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***Abstract*:** Colorectal cancer (CRC) is the second most lethal cancer worldwide and the prognosis of metastatic CRC (mCRC) remains poor. Recent advancements in translational research have led to the identification of several new therapeutic targets and improved the treatment outcome of patients with tumours harbouring *BRAF* V600E mutation, (*HER2*) ErBB2 alterations, *NTRK* gene fusions and *KRAS*(G12C) mutation. Improved understanding towards the mechanism of resistance to targeted therapy such as anti-epidermal growth factor receptor antibodies and the evolving role of therapeutic monitoring with circulating tumour DNA (ctDNA) has enabled the longitudinal tracking of clonal evolution during treatment and the individualization of subsequent treatments. To broaden the community-based implementation of precision oncology in directing targeted therapies for patients with gastrointestinal cancers including mCRC, the feasibility of 'Master Protocols' that utilizes ctDNA-based genotyping platforms is currently being evaluated. Such protocols encompass both observational and interventional clinical trials of novel targeted therapies conducted within a large clinical trial network. In this review, we will discuss the latest developments in targeted therapies, and therapeutic strategies for overcoming acquired drug resistance in patients with mCRC.

Keywords: BRAF inhibitor, colorectal cancer, EGFR inhibitor, personalized medicine, precision oncology, targeted therapy

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Introduction

Colorectal cancer (CRC) is the third most prevalent (10%) and second most lethal (9.4%) cancer worldwide.[1] Although only 21% have distant metastasis on initial presentation,[2] 20–35% of resected stage II–III patients experience recurrence within 5 years, with most of them being distant recurrence.[3] Over the last two decades, different treatment modalities for metastatic CRC (mCRC) have emerged. The combination of fluoropyrimidines with oxaliplatin or irinotecan has increased median overall survival (OS) to nearly 20 months.[4,5] The addition of anti-vascular endothelial growth factor (VEGF) in unselected patients and anti-epidermal growth factor receptor (EGFR) in *KRAS* (Kirsten rat sarcoma virus)-wild type (wt) patients further prolonged OS.[6,7] The integration of multimodal therapy including surgical resection, radiofrequency ablation, targeted radiotherapy and notably, neoadjuvant chemotherapy in liver metastasis has made oligometastatic disease a potentially curable condition in carefully selected patients. With all the aforementioned advances, survival for mCRC has improved over the past decade from a 5-year survival of 10.3% in 1996–2003 to 14.3% in 2010–2016.[8]

The uniqueness of CRC is characterized by its inter-metastatic and intra-tumour heterogeneity, which contributes to the complexity of the disease.[9] The comprehensive genome-wide profiling of CRC by The Cancer Genome Atlas has allowed a more thorough understanding of the disease.[10] Several novel actionable targets including *BRAF* (serine/threonine-protein kinase B-Raf) V600E mutation, *ErBB2* (V-erb-b2 erythroblastic leukaemia viral oncogene homolog 2) alterations, *KRAS*(G12C) mutation and gene fusions such as *NTRK*

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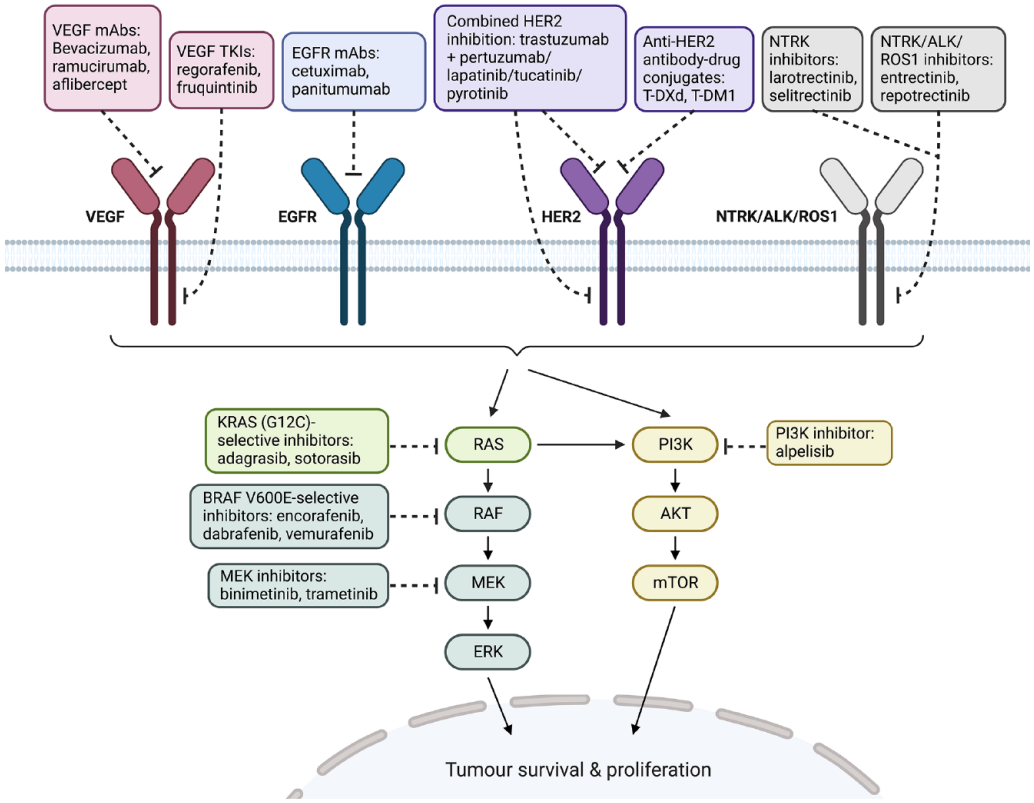

Figure 1. Selected summary of actionable targets and their targeted therapies with demonstrable clinical efficacy in mCRC.
Source: Adapted from 'HER2 Signalling Pathway', by BioRender.com (2022). Retrieved from https://app.biorender.com/biorender-templates.
ALK, anaplastic lymphoma kinase; BRAF, serine/threonine-protein kinase B-Raf; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; HER2, human epidermal growth factor receptor 2; KRAS, Kirsten rat sarcoma virus; mAbs, monoclonal antibodies; mCRC, metastatic colorectal cancer; MEK, mitogen-activated protein kinase kinase; mTOR, mammalian target of rapamycin; NTRK, neurotrophic tyrosine receptor kinase; PI3K, phosphoinositide 3-kinase; ROS1, c-ros oncogene 1; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan or DS-8201; TKIs, tyrosine kinase inhibitors; VEGF, vascular endothelial growth factor.

(neurotrophic tyrosine receptor kinase), *ALK* (anaplastic lymphoma kinase) and *ROS-1* (c-ros oncogene 1) have been identified over the past decade (Figure 1). The identification of tumour-sidedness as a predictive biomarker of response to EGFR antibodies and the use of circulating tumour DNA (ctDNA) in guiding the re-challenge of EGFR antibodies have significantly influenced the contemporary management of mCRC. In this review, we will discuss the recent advancements in targeted therapies and their impact on the clinical outcome of patients with mCRC.

EGFR inhibition

The role of the EGFR receptor tyrosine kinase (RTK) in CRC carcinogenesis has been recognized for more than 30 years – by downstream signalling through mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K)/AKT and JAK (Janus kinase)/STAT (activator of transcription) pathways, EGFR promotes cancer cell survival and proliferation and was recognized as an actionable target in mCRC.[11] The EGFR monoclonal antibodies cetuximab and panitumumab have been shown to be efficacious across different lines of therapy for RAS-wt mCRC.[12,13]

In first-line treatment for patients with RAS-wt mCRC, the addition of cetuximab or panitumumab improved progression-free survival (PFS) [hazard ratio (HR) = 0.65, $p < 0.00001$], OS (HR = 0.83, $p = 0.07$) and objective response rates (ORR) (relative risk = 1.55, $p = 0.0009$) compared with chemotherapy alone.[14] To cite an example,

the addition of panitumumab to FOLFOX4 increased median OS from 19.7 to 23.9 months ($p = 0.072$).[7] EGFR antibodies have been combined with doublet or triplet regimens containing 5-fluorouracil (5-FU), leucovorin, oxaliplatin and/or irinotecan with manageable safety profiles and efficacy.[15–17] However, oral capecitabine is not a recommended chemo-backbone for EGFR antibodies due to overlapping toxicity and reduced survival benefit.[18] The TRIPLETE phase III study randomized patients to panitumumab in combination with either the modified (m)FOLFOXIRI or mFOLFOX regimen. The result has been recently reported, showing that four-drug regimen did not improve PFS (12.7 *versus* 12.3 months, $p = 0.277$) nor ORR (73% *versus* 76%, $p = 0.526$) over the three-drug regimen, with notably higher rates of grade 3–4 adverse events (AEs) (69% *versus* 57%).[19]

Maintenance therapy with EGFR antibodies has been shown to improve PFS in some studies[20,21]; however, it remains unclear whether they should be used alone or with fluoropyrimidines.[22,23] While fluoropyrimidine-based therapy has been used as maintenance for mCRC, the recent PANAMA trial has established a modest PFS improvement with the addition of panitumumab to maintenance fluoropyrimidine over fluoropyrimidine alone (8.8 *versus* 5.7 months, $p = 0.014$).[24]

Downstream activation of the MAPK pathway, including alterations in *EGFR, KRAS, NRAS* (neuroblastoma RAS viral oncogene homolog) and *BRAF*, is the main cause of both innate and acquired resistance to EGFR antibodies in mCRC.[25,26] *KRAS, NRAS* and *BRAF* V600E mutations were found in 35.9%, 4.1% and 6.8% of mCRC patients, respectively,[27] and studies have shown that mutations in *KRAS* exon 2–4, NRAS exon 2–4 and *BRAF* V600E confer resistance to EGFR antibodies.[12,28] Current guidelines therefore support extended *RAS* and *BRAF* mutation testing upon the diagnosis of mCRC.[29] In addition, genetic alterations, including *HER2* amplification, *PIK3CA* mutation and *PTEN* (phosphatase and tensin homolog) inactivation, have all been linked to EGFR antibody resistance in clinical studies.[30–35]

EGFR antibody re-challenge

Re-challenge therapy is defined as retreatment following a treatment-free interval of the particular drug in question, where the tumour displayed initial response/disease control and then developed resistance during treatment.[36] The strategy was first reported by Santini *et al.*, where 39 patients were re-challenged with irinotecan-based cetuximab therapy after initial response/disease stabilization and then clinical resistance to the same therapy.[37] Subsequently, several phase II trials were conducted and demonstrated that re-challenging patients with cetuximab or panitumumab have modest benefits in later lines of therapy (Table 1).[38–43] However, the factors that may predict benefit from EGFR antibody re-challenge are needed as the ORR ranged from 2.9% to 21% in these trials[38–41] and there are other competing options in later lines such as regorafenib.[44] The CRICKET study investigated the use of ctDNA in detecting the presence *RAS* and *BRAF* mutation in patients prior to re-treatment with EGFR antibodies, and found that 12 out of the 25 evaluable patients had detectable *RAS* mutations in ctDNA. Patients without detectable *RAS* mutation in ctDNA had significantly longer PFS compared with those with detectable *RAS* mutations following EGFR antibody re-challenge (4.0 *versus* 1.9 months, $p = 0.03$).[38] This finding supports the hypothesis that *RAS*-wt cancer clones that are sensitive to EGFR antibodies may be restored during a period of treatment break.

The JACCRO CC-08 study showed that the treatment-free intervals between EGFR antibody treatment may affect subsequent response to EGFR antibody re-challenge. Patients with EGFR antibody-free intervals of more than 1 year had prolonged median PFS (4.6 *versus* 2.1 months) and OS (14.1 *versus* 6.3 months) compared with their counterparts.[40] However, some studies have yielded conflicting results.[39,42] In the recently reported CHRONOS study, 36 (69%) out of 52 patients were found to be *RAS*/*BRAF*-wt in ctDNA analyses. An ORR of 30% was achieved among the 27 evaluable patients – which is relatively high compared with historic rates of up to 20% as discussed previously. Interestingly, after disease progression with anti-EGFR re-challenge, de novo MET amplification was detected on ctDNA in three patients, suggesting MET amplification being a potential therapeutic target for patients progressing on anti-EGFR therapy. The authors have also noted that the presence of resistance-conferring mutations and objective responses were independent of the EGFR antibody-free interval, suggesting that ctDNA maybe more effective in selecting patients for re-challenge therapy.[42]

Table 1. Selected past and ongoing prospective clinical trials on anti-EGFR re-challenge in metastatic CRC.

Trial	Phase	Sample size	Treatment arm(s)	Previous treatments	ORR (%)	mPFS (months)	mOS (months)	Remarks
CRICKET[38]	II	28	Cetuximab + irinotecan	1L cetuximab + irinotecan-based chemotherapy; 2L bevacizumab + oxaliplatin-based chemotherapy	21	3.4	9.8	ctDNA RAS-wt patients had prolonged mPFS (4.0 *versus* 1.9 months)
E-RECHALLENGE[39]	II	33	Cetuximab + irinotecan	Fluoropyrimidine, oxaliplatin, irinotecan, cetuximab and bevacizumab	15.6	2.9	8.6	ctDNA RAS-wt patients had prolonged mPFS (7.0 *versus* 2.9 months)
JACCRO CC-08[40]	II	34	Cetuximab + irinotecan	1L cetuximab + irinotecan/oxaliplatin-based chemotherapy; 2L oxaliplatin/irinotecan-based chemotherapy	2.9	2.4	8.2	Patients with anti-EGFR-free interval >372 days had prolonged mPFS (4.6 *versus* 2.1 months) and mOS (14.1 *versus* 6.3 months)
JACCRO CC-09[41]	II	25	Panitumumab + irinotecan	1L panitumumab + FOLFOX/FOLFIRI; 2L bevacizumab/ panitumumab + chemotherapy	8.3	3.1	8.9	Patients with anti-EGFR-free interval >372 days had prolonged mPFS (4.42 *versus* 2.51 months) and mOS (15.84 *versus* 7.33 months)
CHRONOS[42]	II	27	Panitumumab	Previous anti-EGFR in 1L (63%), 2L (15%) or >2L (22%)	30	3.7	–	Only patients with ctDNA RAS/ BRAF/EGFR-wt were enrolled
CAVE[43]	II	77	Cetuximab + avelumab	1L anti-EGFR + chemotherapy, at least one >1L therapy	7.8	3.6	11.6	ctDNA *RAS/ BRAF*-wt patients had prolonged mPFS (4.1 *versus* 3.0 months) and mOS (17.3 *versus* 10.4 months); 4% were MSI-high tumours
REMARRY & PURSUIT[45] (UMIN000036424) (jRCTs031190096)	II	50	Panitumumab + irinotecan	Fluoropyrimidine, oxaliplatin, irinotecan and anti-EGFR	14	3.6	–	ctDNA RAS-wt patients had better ORR (16% *versus* 0%); patients with anti-EGFR-free interval >365 days had better ORR (44.4% *versus* 7.3%)

(Continued)

Table 1. (Continued)

Trial	Phase	Sample size	Treatment arm(s)	Previous treatments	ORR (%)	mPFS (months)	mOS (months)	Remarks
FIRE-4 (NCT02934529)	III	550	Cetuximab + irinotecan-based chemotherapy *versus* regorafenib	1L cetuximab + FOLFIRI; 2L bevacizumab + FOLFOX	–	–	–	–
CAPRI II GOIM (NCT05312398)	II	200	Cetuximab + irinotecan *versus* TAS-102 or regorafenib	1L cetuximab + FOLFIRI; 2L cetuximab + FOLFOX *versus* bevacizumab + FOLFOX	–	–	–	Patients will be selected for suitable second- and third-line therapies according to ctDNA RAS-BRAF status
PULSE (NCT03992456)	II	120	Panitumumab *versus* TAS-102 or regorafenib	–	–	–	–	ctDNA analysis included
PARERE (NCT04787341)	II	214	Panitumumab followed by regorafenib *versus* regorafenib followed by panitumumab	1L anti-EGFR-based therapy; previous 5-FU, oxaliplatin, irinotecan and anti-angiogenics	–	–	–	Only ctDNA *RAS/BRAF*-wt patients will be enrolled
VELO (EudraCT: 2018-001600-12)	II	112	Panitumumab + TAS-102 *versus* TAS-102 alone	–	–	–	–	–
NCT03524820	II	60	Cetuximab ± chemotherapy	1L anti-EGFR + chemotherapy; other 2L treatments	–	–	–	–

ctDNA, circulating tumour DNA; EGFR, epidermal growth factor receptor; mOS, median overall survival; mPFS, median progression-free survival; MSI, microsatellite instability; ORR, objective response rate; wt, wild type; 1L, first-line therapy; 2L, second-line therapy; 5-FL, 5-fluorouracil.

Several ongoing phase II/III trials will confirm the benefit of re-challenge therapy in *RAS*-wt ctDNA-selected patients while looking further into other molecular predictors (Table 1) (UMIN000036424, jRCTs031190096, NCT02934529). In the preliminary reports of the REMARRY and PURSUIT trials (UMIN000036424, jRCTs031190096), although EGFR antibody re-challenge did not meet the pre-specified primary endpoint of ORR (14%), the results confirmed the utility of ctDNA and EGFR antibody-free interval in selecting patients for re-challenge therapy. Five out of 31 patients (16%) with ctDNA *RAS*-wt at progression during prior EGFR antibody therapy responded to re-challenge, while none of the seven patients who were ctDNA *RAS*-mt responded ($p = 0.25$). Furthermore, patients with an EGFR antibody-free interval of over 1 year had a significantly higher ORR (44.4% *versus* 7.3%, $p = 0.0037$).[45] The ongoing FIRE-4 phase III randomized controlled trial (RCT) will compare the efficacy of re-challenge cetuximab-chemotherapy treatment with regorafenib in the third-line setting, following disease progression after first-line cetuximab plus FOLFIRI and second-line bevacizumab plus FOLFOX (NCT02934529).

VEGF inhibition

The anti-VEGF antibody bevacizumab was one of the first biologics alongside with cetuximab approved for the indication of treating patients with mCRC, regardless of the *RAS* mutational status.[46,47,48] Several meta-analyses have demonstrated that bevacizumab could be combined safely with doublet chemotherapy comprising of 5-FU, capecitabine, oxaliplatin and/or irinotecan with survival gains as first-line treatment.[49–51] In a pooled meta-analysis of 1697 patients from five

RCTs which investigated the optimal chemotherapy regimen to be combined with bevacizumab, the triplet FOLFOXIRI backbone was found to have longer PFS (HR = 0.74, $p < 0.001$) and OS (HR = 0.81, $p < 0.001$) compared with FOLFIRI when combined with bevacizumab, at the expense of higher rates of toxicity such as neutropenia, febrile neutropenia, mucositis and diarrhoea.[51] First-line FOLFOXIRI with bevacizumab is now one of the standard therapeutic options in medically fit patients with mCRC.[29] The role of bevacizumab in maintenance therapy remains unclear. Although significant PFS benefits were observed in bevacizumab plus fluoropyrimidine in some studies,[52,53] an individual-patient data meta-analysis showed that the PFS difference between the bevacizumab monotherapy and observation group was limited to less than 1 month (9.6 *versus* 8.9 months, $p < 0.0001$).[54] Since fluoropyrimidine–bevacizumab and fluoropyrimidine alone are regarded as some of the popularly used maintenance approaches following initial chemotherapy, the ongoing BEVAMAINT study will formally compare the two strategies (NCT04188145).

In second-line therapy, VEGF inhibitors including the anti-VEGFR2 antibody ramucirumab and the recombinant fusion protein aflibercept combined with FOLFIRI demonstrated survival benefits.[55,56] Bevacizumab is the only agent to date which have been shown prospectively to be safe and effective when combined with oral capecitabine-based regimen.[57–59] In the AXEPT non-inferiority trial, 650 Asian patients were randomized to mXELIRI (or FOLFIRI) with or without bevacizumab. The median OS of the mXELIRI group was non-inferior to the FOLFIRI group (16.8 *versus* 15.4 months), and the mXELIRI group had an encouraging safety profile with numerically lower incidences of grade 3–4 AEs compared with the latter (54% *versus* 72%).[60] The oral tyrosine kinase inhibitor (TKI) fruquintinib selectively binds to VEGFR1–3 and the recent FRESCO-2 international RCT has demonstrated fruquintinib as a viable option for refractory mCRC. Compared with placebo, fruquintinib improved OS (7.4 months *versus* 4.8 months, $p < 0.001$) and PFS (3.7 months *versus* 1.8 months, $p < 0.001$) with tolerable safety profile (grade ⩾3 AE 62.7% *versus* 50.4%).[61]

Patient selection for targeted therapy in the first-line treatment of microsatellite stable metastatic CRC: new studies

Primary tumour sidedness

A meta-analysis of the FIRE-3, CALGB/SWOG 80405 and PEAK trials compared the efficacy of first-line chemotherapy with EGFR antibodies or with bevacizumab, after stratifying patients into having left-sided or right-sided *RAS*-wt primary tumours. The use of EGFR antibodies was more favourable in patients with left-sided primaries in terms of OS and ORR after exclusion of *BRAF*-mutant patients, whereas bevacizumab demonstrated better PFS in right-sided tumours.[62] In another meta-analysis of the CRYSTAL and PRIME trials, only patients with left-sided tumours significantly benefited from first-line usage of chemotherapy and EGFR antibodies compared with chemotherapy alone.[62] The DEEPER study showed that the depth of response to cetuximab when combined with mFOLFOXIRI was significantly superior than when combined with bevacizumab in patients with left-sided primary tumours.[63] Recently, the PARADIGM trial provided the first ever prospective evidence of panitumumab in providing greater OS benefit and higher objective response over bevacizumab when combined with mFOLFOX6 in both the intention-to-treat cohort (all RAS-wt tumours, $n = 802$) and in patients with left-sided primary tumours ($n = 604$). The median OS was 37.9 months *versus* 34.3 months (HR = 0.82, 95% CI: 0.68–0.99, $p = 0.031$), and the ORR was 80.2% *versus* 68.6% favouring the panitumumab plus mFOLFOX6 arm.[64] Anti-EGFR blockade combined with doublet or triplet chemotherapy is therefore considered as the standard of care for patients with left-sided, RAS-WT mCRC. However, the optimal treatment for right-sided tumours is yet to be defined, and the prognosis of this population remains poor. As studies showed that the benefit from bevacizumab therapy is independent of primary tumour sidedness,[65,66] bevacizumab plus chemotherapy is a reasonable treatment option for right-sided RAS-wt tumours.

Optimal treatment sequence

Preclinical studies have suggested that VEGF expression increases upon onset of anti-EGFR resistance,[67] whereas exposure to bevacizumab

induces elevated VEGF-A levels which activates STAT-3 and VEGFR2 signalling, leading to resistance to cetuximab.[68] A number of retrospective studies reported consistent findings, as first-line chemotherapy with EGFR antibody followed by second-line chemotherapy with anti-VEGF achieved more favourable survival benefits than the reverse treatment sequence.[69–71] The STRATEGIC-1 phase III trial is a RCT which investigated the impact of drug sequencing on the primary endpoint of disease control in treatment-naïve patients with mCRC. This study randomized 263 patients with RAS/BRAF-wt mCRC, to FOLFIRI-cetuximab followed by mFOLFOX6-bevacizumab (Arm A), or first-line oxaliplatin-based regimen in a 'stop-and-go' manner with bevacizumab, followed by second-line FOLFIRI-bevacizumab and third-line anti-EGFR with or without irinotecan (Arm B). Although the study did not meet its primary endpoint, the findings were consistent with the FIRE-3 and PARADIGM study since the use of EGFR antibody-based chemotherapy in the first line (arm A) resulted in higher tumour responses and a trend for better OS (37.8 *versus* 34.4 months, $p = 0.121$).[72] The ongoing phase III CR-SEQUENCE study will evaluate the optimal treatment sequence with panitumumab and bevacizumab combined with chemotherapy in RAS-wt left-sided tumours (NCT03635021).

Uncommon molecular groups in metastatic CRC

BRAF *V600E mutation*

BRAF mutations are found in 7.1–12.5% of mCRCs[27,73–75] and the majority are V600E hotspot mutations.[73] The mutation is thought to play a fundamental role in the serrated pathway of CRC pathogenesis – it is closely linked to epigenetic hypermethylation changes of promotor CpG islands associated with silencing of tumour suppressors. CpG island methylation, along with BRAF downstream MAPK pathway signalling which exert synergistic effects in *BRAF*-mt tumours.[76] *BRAF* mutations are mutually exclusive from *RAS* mutations,[77] and are more commonly found in microsatellite instability (MSI)-high tumours.[74] *BRAF*-mt mCRCs are also associated with poorer survival outcomes and several unfavourable characteristics, such as proximal tumour location, higher T staging, poor differentiation and higher rates of peritoneal and distant lymph node metastases.[46,74,78] Compared with *BRAF* V600E mutations, non-V600E mutations tend to confer better prognosis with distinct clinicopathological characteristics.[79]

Conventional first-line therapy for BRAF *mutants.* Since *BRAF* activates the MAPK pathway downstream of EGFR, *RAS*-wt/*BRAF*-mt tumours are less responsive to EGFR inhibitors.[28] The FIRE-4.5 (AIO KRK-0116) study is a randomized phase II study of *BRAF*-mt/*RAS*-wt patients who had treatment-naïve mCRC, in which 108 patients were randomized to FOLFOXIRI with cetuximab or with bevacizumab. The bevacizumab arm was associated with better ORR (66.7% *versus* 52.0%) and median PFS (8.3 *versus* 5.9 months) compared with the cetuximab group.[80] Bevacizumab plus triplet or doublet chemotherapy should therefore be considered the preferred option for the first-line treatment of *BRAF*-mt mCRC before the era of *BRAF* inhibition.[29,48]

However, it is debatable whether bevacizumab plus triplet FOLFOXIRI chemotherapy brings more benefit than doublet FOLFOX/FOLFIRI chemotherapy in *BRAF*-mt patients. The TRIBE study contributed the first piece of evidence to the controversy, as a small subgroup analysis with 28 *BRAF*-mt patients of the phase III TRIBE study showed a trend of longer OS in bevacizumab plus FOLFOXIRI *versus* FOLFIRI.[46] The analysis was however underpowered, and the results were later challenged by an individual patient data meta-analysis from five studies, which showed no difference in survival of bevacizumab with triplets *versus* doublets in *BRAF* mutants (HR = 1.11). In addition, FOLFOXIRI plus bevacizumab was associated with significantly higher rates of grade 3–4 gastrointestinal and haematological AEs; thus, the authors concluded that FOLFOX, instead of FOLFOXIRI, should be regarded as the standard-of-care chemotherapy regimen to combine with bevacizumab in *BRAF*-mt mCRC patients.[51] The recent result from the CAIRO5 study adds to the controversy by taking into account of tumour-sidedness. In this study, 294 patients with *BRAF* V600E-mt and/or right-sided primary tumours were randomized to receive either first-line FOLFOX or FOLFIRI plus bevacizumab (arm A) or FOLFOXIRI plus bevacizumab (arm B). Patients in arm B had significantly longer PFS (9.0 *versus* 10.6 months, $p = 0.02$) and ORR (32.0% *versus* 52.1%, $p < 0.001$) at the expense of more frequent grade $\geqslant 3$ toxicities (58.5% *versus* 75.0%, $p = 0.003$).[81] FOLFOXIRI-bevacizumab may therefore be considered in

right-sided BRAF-mt patients if intensified chemotherapy could be tolerated.

Targeting the MAPK pathway. Unlike melanoma, BRAF inhibitor has a modest single agent response rate of 5% in patients *BRAF*-mt mCRC.[82] This may be in part due to MAPK activation as a potential resistance mechanism against BRAF inhibition, thus prompting the use of multi-drug combinations targeting BRAF, EGFR and the MAPK pathway.[83] The role of dual BRAF and EGFR inhibition in *BRAF*-mt mCRC patients was first demonstrated in a pilot trial by Yaeger *et al.*,[84] where two out of 15 patients had partial responses to vemurafenib and panitumumab. Several studies further assessed the efficacy and safety profile of different combinations of BRAF, EGFR and MEK inhibitors (Table 2).[85–90] The phase III BEACON trial was the first to demonstrate the superiority of encorafenib-based, chemotherapy-free regimens over the standard EGFR-antibody plus chemotherapy in patients with previously treated *BRAF*-mt mCRC. Both the doublet (encorafenib plus cetuximab) and triplet (encorafenib, cetuximab plus binimetinib) were superior to the control arm (cetuximab plus irinotecan-based chemotherapy) in terms of median OS (9.3 months *versus* 5.9 months) and ORR (26.8% for triplet, 19.5% for doublet and 1.8% for control arm). Although the triplet group had higher rates of grade ⩾3 AEs (65.8%) than the doublet group (57.4%), both arms showed similar toxicity profiles compared with the control group (64.2%).[85] Furthermore, a recent study reported that patients with tumours of the Consensus Molecular Subtype 4 (CMS4) and *BRAF* mutant 1 (BM1) having higher response rates with the triplet than the doublet therapy.[91] Therefore, while it is generally agreed that the doublet (encorafenib plus cetuximab) is currently preferred over the triplet regimen given the similar impact on OS, certain high-risk subgroups may potentially be more suitable for triplet therapy.

Table 2. Selected past and ongoing prospective clinical trials on targeted therapy and immunotherapy in *BRAF* V600E metastatic CRC.

Trial	Phase	Sample size	Treatment arm(s)	Line of treatment (and MSI status)	ORR (%)	mPFS (months)	mOS (months)
Combined BRAF, EGFR ± MEK inhibition							
Yaeger *et al.*[84]	I	15	Vemurafenib + panitumumab	⩾2L	13	3.2	7.6
Corcoran *et al.*[89]	I	20	Dabrafenib + panitumumab	⩾1L	10	3.5	13.2
		91	Dabrafenib + panitumumab + trametinib	⩾1L	21	4.2	9.1
BEACON CRC[85]	III	220	Encorafenib + cetuximab (*versus* cetuximab + irinotecan-based chemotherapy)	⩾2L	19.5 (*versus* 1.8)	4.3 (*versus* 1.5)	9.3 (*versus* 5.9)
		224	Encorafenib + cetuximab + binimetinib (*versus* cetuximab + irinotecan-based chemotherapy)	⩾2L	26.8 (*versus* 1.8)	4.5 (*versus* 1.5)	9.3 (*versus* 5.9)
ANCHOR CRC[87] (NCT03693170)	II	92	Encorafenib + cetuximab + binimetinib	1L	47.8	5.8	17.2
BREAKWATER (NCT04607421)	III	765 (in 3 arms)	Encorafenib + cetuximab *versus* encorafenib + cetuximab + FOLFOX (*versus* bevacizumab + FOLFOX/FOLFIRI/XELOX)	1L	–	–	–
Immunotherapy plus BRAF doublet inhibition							
Corcoran *et al.*[92]	II	20	Dabrafenib + trametinib + spartalizumab	Unspecified (MSI-unselected)	35	–	–
Morris *et al.*[93]	II	23	Encorafenib + cetuximab + nivolumab	⩾2L (MSS patients)	48	7.4	15.1
SWOG 2107 (NCT05308446)	II	84 (in 2 arms)	Encorafenib + cetuximab + nivolumab (*versus* encorafenib + cetuximab)	⩾2L (MSS patients)	–	–	–

(Continued)

Table 2. (Continued)

Trial	Phase	Sample size	Treatment arm(s)	Line of treatment (and MSI status)	ORR (%)	mPFS (months)	mOS (months)
SEAMARK (NCT05217446)	II	104 (in 3 arms)	Encorafenib + cetuximab + pembrolizumab (*versus* pembrolizumab alone)	1L (MSI-high patients)	–	–	–
Combined BRAF, EGFR and PI3K inhibition							
Tabernero *et al.*[94]	II	52	Encorafenib + cetuximab + alpelisib (*versus* encorafenib + cetuximab)	⩾2L	27 (*versus* 22)	5.4 (*versus* 4.2)	15.2 (*versus* not reached)
BRAF and EGFR inhibition plus irinotecan							
SWOG S1406[90]	II	50	Vemurafenib + cetuximab + irinotecan *versus* cetuximab + irinotecan	2L/3L	17 (*versus* 4)	4.2 (*versus* 2.0)	9.6 (*versus* 5.9)

BRAF, serine/threonine-protein kinase B-Raf; CRC, colorectal cancer; EGFR, epidermal growth factor receptor; MEK, mitogen-activated protein kinase kinase; mOS, median overall survival; mPFS, median progression-free survival; MSI, microsatellite instability; MSS, microsatellite stability; ORR, objective response rate; PI3K, phosphoinositide 3-kinase.

The phase III BREAKWATER (NCT04607421) and phase II single-arm ANCHOR CRC[87] (NCT03693170) studies are two notable clinical trials which evaluate BRAF-EGFR and/or MEK inhibitors in the first-line treatment of *BRAF*-mt mCRC. In the ANCHOR CRC study, an ORR of 47.8% was observed in the 92 evaluable patients who received encorafenib–binimetinib–cetuximab, with a median OS of 17.2 months a grade ⩾3 AEs rate of 69.5%.[87] Apart from encorafenib-based therapies, regimens including other BRAF inhibitors such as dabrafenib–panitumumab–trametinib, dabrafenib–panitumumab and vemurafenib–cetuximab–irinotecan have also demonstrated increased survival and tumour response rates in clinical studies, at the expense of higher toxicities.[89,90] Encorafenib-based regimens have been included in the latest NCCN guideline (June 2022), but not the other regimens at this juncture.[29]

Other strategies for BRAF-mutant mCRC. Recently, the addition of programmed death receptor-1 (PD-1) inhibitor to BRAF-inhibitors has been investigated in several trials, as preclinical studies found that the combination demonstrates down-regulation of mismatch repair (MMR) and up-regulation of error-prone polymerases, which induces DNA damage and hypermutability, and ultimately triggers a proficient MMR-to-dMMR phenotypic switch.[95] A single-arm phase II study demonstrated an ORR of 35% with combined dabrafenib, trametinib and the PD-1 antibody spartalizumab in 20 patients with *BRAF* V600E-mt microsatellite status-unselected patients.[92] In another phase I/II trial, 23 treatment refractory microsatellite stable (MSS) *BRAF*-mt patients were treated with encorafenib, cetuximab and nivolumab and 48% attained radiographic response.[88] Based on these results, the phase II SWOG 2107 multi-centre RCT has is evaluating encorafenib plus cetuximab with or without nivolumab in chemo-refractory *BRAF*-mt/MSS patients (NCT05308446), while the phase II SEAMARK study will evaluate encorafenib plus cetuximab with or without pembrolizumab in treatment-naïve *BRAF*-mt/MSI-H patients (NCT05217446).

As PI3K/AKT activation has also been identified as a mechanism of acquired resistance to *BRAF* inhibitors in preclinical studies,[96] a phase II trial compared the triplet encorafenib–cetuximab–alpelisib with the doublet (encorafenib–cetuximab) in patients with chemotherapy-refractory *BRAF*-mt mCRC. The addition of PI3K inhibitor alpelisib resulted in a non-significant trend of higher median PFS from 4.2 to 5.4 months ($p = 0.064$) and confirmatory studies are needed.[94]

HER2 alterations

HER2 or *ErBB2* amplification was one of the earliest-identified therapeutic markers of solid tumours. It is found in around 1.1–5.8% of patients with mCRC especially in *KRAS*/*BRAF*-wt patients.[97–102] *ErBB2* amplification leads to *HER2 RTK* overexpression, it is then activated by dimerization with another receptor of the ErbB family, resulting in transphosphorylation of tyrosine residues within the cytoplasmic domain, thus leading to downstream signal transduction along the MAPK, PI3K/AKT and JAK/STAT pathway.[103] In mCRC, *HER2* overexpression is

associated with left-sided primary, more frequent lung metastases and higher number of metastases.[30,97,98]

Combined HER2 inhibition. Unlike HER2-positive breast cancer, trastuzumab alone is ineffective in HER2-altered mCRC. The HERACLES study is the first to evaluate dual anti-HER2 strategies in HER-2 altered mCRC using trastuzumab and the TKI lapatinib.[104] Among the 32 evaluable HER2-positive mCRC patients heavily pre-treated with chemotherapy and EGFR inhibitors, the respective median PFS and OS were 4.7 and 10 months, while the ORR was 28%, which is seemingly better than the reported activity of standard third-line therapies such as regorafenib or TAS-102.[105] Central nervous system (CNS) recurrence occurred in 19% of the patients, compared with historic rates of 1–4% in mCRC patients. The higher incidence of CNS metastasis maybe due to the propensity of *ErBB2* amplification driving CNS spread, or the limited intracranial activity of anti-HER2 therapies.[105]

Other combinations of anti-HER2 therapies have also been investigated in recent years (Table 3). As preclinical evidence demonstrated greater specificity of tucatinib to HER2 compared with lapatinib,[106] the efficacy of tucatinib–trastuzumab in pre-treated patients has been investigated in the phase II MOUNTAINEER study, which reported an ORR of 38.1% and disease control rate (DCR) of 71.4% at a median follow-up of 20.7 months. Median PFS and OS were 8.2 and 24.1 months, respectively, among the 86 patients. Diarrhoea was the most common treatment-related AE (TRAE) of the combination, yet grade 3 diarrhoea were noted in only 3% of the patients.[107]

Table 3. Selected past and ongoing prospective clinical trials on targeted therapy in HER2-positive metastatic CRC.

Trial	Phase	Sample size	Treatment arm(s)	Line of treatment	ORR (%)	mPFS (months)	mOS (months)
Combined HER2 inhibition							
HERACLES[108]	II	32	Trastuzumab + lapatinib	⩾2L	28	4.7	10.0
MOUNTAINEER[107]	II	86	Trastuzumab + tucatinib	⩾2L	38.1	8.2	24.1
HER2-FUSCC-G[109]	II	11	Trastuzumab + pyrotinib	⩾2L	45.5	7.8	15.0
Yuan *et al.*[110]	II	11	Trastuzumab + pyrotinib	⩾1L	27	–	–
MyPathway[111]	II	84	Pertuzumab + trastuzumab	⩾2L	26.2	–	–
TRIUMPH[112]	II	27	Pertuzumab + trastuzumab	⩾2L	30	4.0	10.1
TAPUR[113]	II	28	Pertuzumab + trastuzumab	⩾2L	14	4.0	–
NSABP FC-11[114]	II	21	Neratinib + cetuximab	⩾2L (prior anti-HER2 not allowed)	33	–	–
S1613 (NCT03365882)	II	240 (in 2 arms)	Pertuzumab + trastuzumab *versus* cetuximab + irinotecan	⩾2L	–	–	–
Anti-HER2 antibody–drug conjugates							
HERACLES-B[115]	II	31	Pertuzumab + T-DM1	⩾2L	9.7	4.1	
DESTINY-CRC01[116]	II	53	T-DXd	⩾3L	45.3	6.9	15.5
DESTINY-CRC02 (NCT04744831)	II	122	T-DXd	⩾2L (prior anti-HER2 allowed)	–	–	–

CRC, colorectal cancer; HER2, human epidermal growth factor receptor 2; mOS, median overall survival; mPFS, median progression-free survival; ORR, objective response rate; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan or DS-8201; 2L, second-line therapy; 3L, third-line therapy.

Preliminary reports from two phase II studies on pyrotinib–trastuzumab for HER2-positive mCRC have been published recently.[109,110] Pyrotinib is a novel irreversible TKI targeting HER2 and EGFR and was shown to be more efficacious than lapatinib when combined with capecitabine in metastatic breast cancer.[117] Among the 11 mCRC patients in the HER2-FUSCC-G study, 45.5% responded to pyrotinib–trastuzumab, and ORR was even higher in RAS-wt patients (55.6%). An acceptable grade 3-4 TRAE rate of 36.4% was reported.[109]

The pertuzumab–trastuzumab combination along with chemotherapy is currently considered a first-line option in patients with HER2-positive metastatic breast cancer.[118] While pertuzumab itself inhibits dimerization between HER2 and HER3, it has a synergistic effect when combined with trastuzumab.[119] The ongoing MyPathway study evaluated this combination in 84 *HER2*-amplified mCRC patients and reported an ORR of 26.2%. Notably, only one of 16 (6.3%) *KRAS*-mt patients responded to the combination, suggesting limited activity of pertuzumab–trastuzumab in KRAS-mt tumours.[111] At a median follow-up of 7.3 months, estimated median PFS and OS were 2.9 and 11.5 months, respectively. Grade 3–4 TRAEs were reported in 37% of the patients.[120] The TRIUMPH and TAPUR phase II studies which evaluated the efficacy of pertuzumab plus trastuzumab in *HER2*-amplified/*RAS*-wt mCRC, also reported similar findings to that of the MyPathway study.[112,113]

Anti-HER2 antibody–drug conjugates. Based on the activity of the antibody–drug conjugate trastuzumab–emtansine (T-DM1) in combination with pertuzumab in metastatic breast cancer, the HERACLES-B trial evaluated this three-drug regimen in 31 patients with chemo-refractory HER2-positive mCRC. The trial however did not reach its primary endpoint of ORR (9.7%). It should be noted that since cytotoxic emtansine was included in the regimen, a lower dose of T-DM1 was delivered. The trial reported a median PFS comparable to other anti-HER2 combination therapies (4.1 months) and only 6.5% of patients experienced grade 3 TRAEs.[115]

The DESTINY-CRC01 trial evaluates the efficacy of trastuzumab–deruxtecan (DS-8201/T-DXd) in 86 mCRC patients who had received two or more lines of therapy, among which 53 were HER2-positive, which was defined as immunohistochemistry (IHC) 3+, or 2+ and *in situ* hybridization (ISH)-positive. More than 30% of the cohort had prior anti-HER2 therapy. The HER2-positive cohort showed an impressive ORR of 45.3%, and median PFS and OS were 6.9 and 15.5 months, respectively, at a median follow-up of 62.4 weeks; ⩾Grade 3 TRAEs were however considerably high (65.1%), with the most common ones being haematological and gastrointestinal AEs. Notably, eight patients (9.3%) had interstitial lung disease related to T-DXd, and three of them resulted in treatment-related deaths.[116] Therefore, the phase II DESTINY-CRC02 trial was recently initiated to assess the efficacy of trastuzumab–deruxtecan at two different doses in HER2-overexpressing advanced CRC[121] (NCT04744831).

Predicting anti-HER2 response and resistance. Several trials have identified the magnitude of ErBB2 amplification or HER2 overexpression as a predictive biomarker of anti-HER2 therapy.[108,116,120] The HERACLES trial tested gene copy number by quantitative real-time PCR and identified that patients with tissue copy number (tCN) ⩾9.45 had prolonged PFS.[108] The MyPathway trial measured HER2 tCN by next-generation sequencing and found that more patients with higher copy number responded to pertuzumab–traztuzumab.[120] Subgroup analysis in the DESTINY-CRC01 trial found that patients with IHC 3+ achieved the highest ORR of 57.5%, compared with only 7.7% of patients with IHC2+ and ISH positive.[116]

Molecular analyses of ctDNA samples from the HERACLES trial revealed that the MAPK pathway was involved in resistance to anti-HER2 therapies, as alterations in RAS/RAF were detected in 86% of treatment-refractory patients but only in 14% of responders. *KRAS, BRAF, HER2, EGFR, PIK3CA* and *PTEN* alterations have also been detected at disease progression, which suggest both MAPK and PI3K/AKT pathways playing a role in acquired resistance against anti-HER2 therapy.[122] Similar findings were also documented in the MyPathway trial, as lower ORRs were seen in *KRAS*-mt (8% *versus* 40%) and *PIK3CA*-mt (13% *versus* 43%) patients compared with their counterparts who are wild-typed.[120]

Owing to the relatively small subset of HER2-positive mCRC, clinical research on subsequent treatment strategies following acquired anti-HER2 resistance is limited. However, T-DXd may be potential strategy to overcome acquired

resistance, as responses are seen in patients who had prior anti-HER2 therapy.[116] The main past and ongoing studies on HER2 inhibition are summarized in Table 3.

NTRK and other actionable gene fusions

Although kinase gene fusions represent only about 0.9% of CRCs, they are considered an actionable group of therapeutic targets. The most frequently detected fusions were NTRK fusions, while other fusions including BRAF, RET, FGFR (fibroblast growth factor receptor), ROS1 and ALK. Higher incidences of gene fusions were found in MSI-high and *RAS*/*BRAF*-wt tumours,[123] and the clinical characteristics of patients harbouring these gene fusions are reminiscent of those in *BRAF* mutations, including older age, right-sided primary, higher rates of lymphatic spread and lower rates of liver metastases.[124,125] As current evidence suggests that gene fusions may be both a negative prognostic factor that predict resistance to EGFR inhibitor, novel strategies targeting gene fusions is an attractive field of research to improve the clinical outcomes for this subset of patients.[34,35,124,125]

First-generation NTRK inhibitors. Owing to the scarcity of gene fusions, most trials targeting gene fusions are designed as tumour-agnostic basket trials. Three phase I studies (LOXO-TRK-14001, SCOUT and NAVIGATE) evaluated the efficacy of the NTRK inhibitor larotrectinib in NTRK-positive metastatic non-CNS primary solid tumours and were included in a pooled analysis. Among the eight patients with colon cancer (out of 153 patients with solid tumours), ORR was 50% and median duration of response was 3.7 months. In a safety population of 260 patients treated regardless of kinase fusion status, 13.5% of them developed grade 3–4 TRAEs.[126] In another integrated analysis of three phase I/II studies STARTRK-1, ALKA-372-001 and STARTRK-2 which evaluated the efficacy of the NTRK/ALK/ROS-1 inhibitor entrectinib, among the four patients (out of 54 patients with solid tumours) with CRC, 1 (25%) achieved objective response. In the overall safety-evaluable population comprising of 355 patients, entrectinib toxicity was generally tolerable as only 4% of patients discontinued treatment due to TRAEs.[127] With their established efficacy and safety profile, the US Food and Drug Administration (FDA) has granted approval to larotrectinib and entrectinib for all solid tumours that harbour NTRK gene fusions, and current guidelines also support larotrectinib and entrectinib as subsequent treatment options for NTRK-positive mCRC.[29]

Overcoming resistance with second-generation NTRK inhibitors. Two different mechanisms of resistance to NTRK inhibitors, namely 'on-target' and 'off-target' resistance, have been described. The on-target resistance is the most common mechanism of resistance to the first-generation NTRK inhibitors larotrectinib and entrectinib, which are caused by mutations that decrease binding affinity of NTRK inhibitors to the kinase domain. Notably, the mutations contributing to on-target resistance to NTRK inhibitors are paralogous to mutations found in lung cancer patients resistant to ALK and ROS1 inhibitors.[128,129] To address on-target resistance to NTRK inhibitors, the second-generation NTRK inhibitors selitrectinib (LOXO-195) and repotrectinib (TPX-0005) were designed and are currently being evaluated in phase I/II studies. A phase I study for selitrectinib was initiated following a case report of confirmed PR in an advanced NTRK-positive CRC patient who developed acquired G595R resistance to previous larotrectinib.[130] A tumour agnostic cohort of 31 NTRK-positive patients who failed prior NTRK inhibition was recruited. Among 20 patients with TRK kinase mutations identified, 9 (45%) achieved complete response (CR)/ partial response (PR), and 6 (30%) had stable disease (SD).[131] The NTRK/ALK/ROS1 inhibitor repotrectinib is currently being evaluated in the phase II tumour-agnostic TRIDENT-1 study. To date, among six NTRK-positive patients pretreated with NTRK inhibitors, three (50%) of them achieved objective response.[132,133]

On the other hand, off-target resistance consists of downstream activation of MAPK signalling including *BRAF* V600E and *KRAS* mutations or alterations in other RTKs such as MET (mesenchymal–epithelial transition) amplification.[128,134] These genetic alterations are mostly identified in patients with gastrointestinal cancers and may account for the poorer response to larotrectinib and entrectinib in CRC compared with other cancers.[126,127] Combinations of different TKIs were therefore postulated as a method to overcome off-target resistance.

KRAS(G12C)-selective inhibitors

Treatment for *KRAS*-mt mCRC is considered challenging as they are refractory to anti-EGFR

therapy and no targeted therapy selective against *KRAS* mutations has been approved for mCRC. Numerous efforts to target KRAS have failed due to its high affinity with guanosine-5′-triphosphate and the absence of suitable binding pockets for drugs.[135] However, the *KRAS*(G12C) mutant has been recently found to harbour a cysteine residue suitable for binding of covalent inhibitors, which led to the development of KRAS(G12C)-selective inhibitors.[136] Following the promising results from the phase II portion of the CodeBreaK100 trial, the KRAS(G12C) inhibitor sotorasib (AMG510) was first approved by the FDA for KRAS(G12C)-mutated non-small-cell lung cancer (NSCLC).[137] Since KRAS(G12C) mutation is only present in around 3% of all mCRCs,[138] studies for KRAS(G12C) inhibition in mCRC are currently limited.

Several KRAS(G12C) inhibitors have been evaluated in phase I/II trials. Recently, a pre-specified analysis of the phase II portion of the CodeBreak100 study was released. Among 62 *KRAS*(G12C)-mt patients who received sotorasib, 9.7% and 72.6% achieved PR and SD, respectively. Median PFS and OS were 4.0 and 10.6 months, respectively, and grade ⩾3 TRAEs were seen in only 10% of the patients. The responses are relatively modest compared with patients with NSCLC where an ORR of 37.1%, median PFS of 6.8 months and OS 12.5 months are noted. This discrepancy maybe due to[139] activation of other RTKs including EGFR which may bypass KRAS(G12C) blockade.[140] Given the successful use of dual inhibition of *BRAF* and EGFR in *BRAF*-mt mCRC,[85] concomitant KRAS(G12C) and EGFR blockade has been suggested to overcome resistance to KRAS(G12C) inhibition in CRC.[139] The KRYSTAL-1 multicohort phase I/II study tested this postulation by evaluating the efficacy of adagrasib (MRTX849) alone or in combination with cetuximab in KRAS(G12C) mutant mCRC. In all, 44 and 32 patients were recruited to adagrasib monotherapy group and adagrasib plus cetuximab group, respectively. Both groups exhibited tolerable toxicity profiles. In adagrasib monotherapy group, the ORR and DCR were 19% and 86%, respectively, while the median PFS was 5.6 months. In the cetuximab–adagrasib group, a higher ORR of 46% and 100% DCR were observed, and median PFS was 6.9 months.[141] As adagrasib plus cetuximab demonstrated promising clinical activity, the combination is currently being evaluated in the phase III KRYSTAL-10 trial in the second-line setting (NCT04793958).

Despite these promising results, acquired resistance to adagrasib may be associated with the presence KRAS alterations, mutations along the MAPK and PI3K pathways and gene fusions involving ALK, RET, BRAF, RAF1 and FGFR3.[142] The diversity of mechanisms provides a strong rationale to support the use of KRAS(G12C) inhibitors with other targeted therapies. Of note, the phosphatase Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) has been identified to be a common node that mediates signalling from multiple RTKs to RAS. Co-inhibition of KRAS(G12C) and SHP2 is able to suppress RAS signalling and induce tumour response *in vitro* and *in vivo*.[143] The combination is further investigated in phase I/II trials NCT04185883 (CodeBreaK101), NCT04330664 (KRYSTAL-2) and NCT04699188 (KontRASt-01). Other combinations involving inhibition of EGFR, PD-1 and MEK are also explored in trials NCT04793958 (KRYSTAL-10), NCT03785249 (KRYSTAL-1), NCT04449874 and NCT04185883 (Code BreaK101).

Conclusion and future directions

The treatment paradigm of mCRC has been rapidly evolving over the past few years. Historically, targeted therapies in mCRC are more active when combined with chemotherapy backbones, but recent studies have shown that chemotherapy-free regimens can improve survival in certain molecular subgroups such as patient with *BRAF*-mt and MSI-H mCRC. RAS-mt MSS tumours represent a significant proportion of mCRC, yet most of them could not benefit from targeted therapy (with the possible exception of KRAS(G12D) mutant mCRC) and immunotherapy. Transforming immune-cold to immune hot tumours has therefore come under the spotlight recently, and VEGF inhibitors and/or chemotherapy combined with immune-checkpoint inhibitors maybe an effective method of re-invigorating the immune system. These hypotheses are currently being tested in several phase I-II studies.[144–148]

The advent of ctDNA analysis may further improve the precision of individualizing targeted therapies for patients with mCRC. Current evidence supports the use of ctDNA in tracking clonal resistance against EGFR antibody therapies and therefore should be used to identify patients who are suitable for EGFR antibody re-challenge therapy – as supported by recently

reported European Society of Medical Oncology guideline.[149] The use of ctDNA in tracking clonal resistance against other targeted therapies against BRAF mutation and NTRK fusions needs to be further evaluated, while the role of ctDNA in predicting early response to targeted therapies remains unclear. In early stage CRC, ctDNA has been shown to be a reliable marker in detecting minimal residual disease after surgical resection and identifying patients who may benefit more from adjuvant chemotherapy.[150,151] Furthermore, ctDNA may be very useful in selecting patients for clinical trials given its convenience compared with tissue genotyping, and has been shown to significantly increase trial enrolment rates without compromising treatment efficacy in the SCRUM-Japan GOZILA studies.[152]

The traditional phase I to phase II-III model of clinical trial designs remains to be one of the causes hindering the development of targeted therapy in mCRC. The novel 'Master Protocol' clinical trial design may overcome such limitations, such as umbrella trials that evaluate multiple treatments in one disease. The COLOMATE study is an ongoing, seamless adaptive protocol that primarily uses ctDNA (Guardant 360) to screen patients with secondary resistance to targeted therapies, where patients will be enrolled into three separate clinical trials depending on their ctDNA genotype: Patients with ctDNA showing HER-2 alteration will be enrolled into a study involving tucatinib, trastuzumab and TAS-102; patients with *BRAF* V600E mutation in ctDNA are re-challenged with encorafenib, cetuximab and binimetinib; patients without ctDNA RAS mutations are re-challenged with panitumumab[153] (PULSE study; NCT03992456).

As the number of new targeted therapies are being discovered and evaluated in patients with mCRC, the priorities of research in the next decade should include (1) the development of novel strategies to overcome secondary drug resistance; (2) to refine the detection limit, accuracy and optimal timing of ctDNA in monitoring emerging drug resistance during treatment and in directing subsequent therapies; (3) to evaluate targeted therapies in other clinical settings such as in locally advanced rectal cancer and in the postoperative adjuvant treatment following resection of oligometastases in mCRC; (4) to optimize the safe and effective combination of targeted therapy with immunotherapy and other treatment modalities such as radiotherapy and (5) to evaluate relevance of the CMS subtyping of mCRC in determining response to certain targeted therapies.

Declarations

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Author contribution(s)

Ambrose HN Wong: Conceptualization; Writing – original draft; Writing – review & editing.

Brigette Ma: Conceptualization; Supervision; Writing – original draft; Writing – review & editing.

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