Current challenges in the implementation of precision oncology for the management of metastatic colorectal cancer

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ABSTRACT

Over the last few decades, molecularly targeted agents have been used for the treatment of metastatic colorectal cancer. They have made remarkable contributions to prolonging the lives of patients. The emergence of several biomarkers and their introduction to the clinic have also aided in guiding such treatment. Recently, next-generation sequencing (NGS) has enabled clinicians to identify these biomarkers more easily and reliably. However, there is considerable uncertainty in interpreting and implementing the vast amount of information from NGS. The clinical relevance of biomarkers other than NGS are also subjects of debate. This review covers controversial issues and recent findings on such therapeutics and their molecular targets, including VEGF, EGFR, BRAF, HER2, RAS, actionable fusions, Wnt pathway and microsatellite instability for comprehensive understanding of obstacles on the road to precision oncology in metastatic colorectal cancer.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide, comprising a considerable portion of the disease burden. About half of new cases and deaths occur in Asia, where awareness of CRC has risen in recent times. Approximately 20% of new cases present with distant metastases and 20%–25% of localised cases eventually experience recurrence. Systemic chemotherapy as well as multidisciplinary curative approaches have improved the survival of patients with metastatic CRC (mCRC).

Although the backbone of systemic therapy for mCRC still remains cytotoxic agents, new targets and therapeutics have emerged in the last few decades based on an improved understanding of the biology of CRC. Furthermore, the use of next-generation sequencing (NGS) tests to guide targeted therapy in mCRC has become increasingly prevalent in clinical settings due to falling costs. However, the NGS test results provide a bulk of information that requires careful interpretation. Besides, other biomarkers such as consensus molecular subtype (CMS), circulating tumour DNA (ctDNA), plasma proteins, microRNA and sidedness have been suggested to predict the efficacy of targeted agents. However, the clinical relevance of these markers remains controversial.

This review examines clinical and translational data concerning targeted agents for mCRC and deals with issues regarding the predictive value of various biomarkers.

Anti-angiogenic agents: still the universal answer to mCRC?

Several anti-angiogenic agents such as bevacizumab, aflibercept, ramucirumab and regorafenib, which are designed to bind to vascular endothelial growth factor (VEGF) or VEGF receptors (VEGFR), have been approved for the treatment of mCRC. Patient subgroups who benefit more from anti-VEGF treatment have not been clearly defined based on clinical characteristics or biomarkers. Several translational projects with randomised trials explored mutations in RAS, BRAF, PIK3CA or phosphatidylinositol 3-kinase catalytic alpha polypeptide (PIK3CA) and other biomarkers (table 1). Recently, microsatellite instability (MSI) status and CMS, a transcriptome-based molecular subtype identified by an international consortium study, have been suggested to predict the efficacy of bevacizumab. Briefly, CMS 1 and high MSI (MSI-H) were associated with a clinical benefit from bevacizumab, as opposed to cetuximab, in one of the largest translational sets analysed thus far, the CALGB/SWOG 80405 trial. However, these results were not reproduced in a similar FIRE-3 study, which conducted a head-to-head trial of bevacizumab versus cetuximab. In contrast, the AGITG MAX study showed better progression-free survival (PFS) in patients with CMS 2 or 3 (but not CMS 1) when they were administered bevacizumab.
Table 1 Biomarker studies from randomised trials comparing anti-VEGF or anti-EGFR antibodies to NO targeted therapies for mCRC

| Study (author/year) | Number of patients (analysed for biomarker/ randomised) | Design | Biomarker analysis platform | Analysed markers | Results |
|---------------------|--------------------------------------------------------|--------|----------------------------|------------------|---------|
| AVF2107 (Hurwitz et al, 2009) | 230/813 | 1st line, Bev+IFL vs placebo+IFL | Direct sequencing | KRAS mutation | PFS-related benefit of Bev similar for KRAS-wt (HR 0.41, p=0.00008) and KRAS-mt (HR 0.83, p=0.00011) | |
| VELOUR (W Yapati et al, 2017) | 482/1226 | 2nd line, afibercept+FOLFIRI vs placebo+FOLFIRI | NGS | Extended RAS, BRAF mutation and transcriptome | Non-significant trend of OS-related benefit with afibercept for BRAF mt (interaction p=0.08) and BRAF mt-like RNA signature (interaction p=0.2) |
| AGITG MAX (Price et al, 2015) | 280/471 | 1st line, bevacizumab+CTx vs CTx | Pyrosequencing | RAS, PIK3CA mutation | None were prognostic or predictive of bevacizumab outcome |
| AGITG MAX (Mooi et al, 2018) | 237/471 | 1st line, bevacizumab+CTx vs CTx | Almac Xcel microarray CMS | Benefit of bevacizumab in terms of PFS in CMS2 (HR 0.54, 95% CI 0.39 to 0.82, interaction p=0.05) and CMS3 (HR 0.35, 95% CI 0.14 to 0.86, interaction p=0.04 in multivariate analysis) |
| RAISE (Tabernero et al, 2018) | 894/1072 | 2nd line, ramucirumab+FOLFIRI vs placebo+FOLFIRI | Dual-monoclonal sandwich immunoassay | VEGF-C, VEGF-D, soluble VEGFR-1, soluble VEGFR2 and soluble VEGFR-3 | High VEGF-D level (≥115 pg/mL) predicted benefit from OS (HR 0.73, 95% CI 0.60 to 0.89, interaction p=0.0005) and PFS (HR 0.62, 95% CI 0.52 to 0.74, interaction p=0.0001) |
| RAISE (Yoshino et al, 2019) | 912/1072 | 2nd line, ramucirumab+FOLFIRI vs placebo+FOLFIRI | Multiplex qPCR (Modaplex system, Qiagen) | RAS, BRAF mutation | No treatment-by-RAS/BRAF mutation status interaction (p=0.523 for OS, 0.655 for PFS), but numerically good OS in BRAF with ramucirumab (HR 0.54, p=0.103) |
| CORRECT (Tabernero et al, 2015) | 503/760 (genetic biomarker) 611/760 (protein biomarker) | 3rd line, regorafenib vs placebo | BEAMing of plasma DNA FoundationOne panel for tumour tissue, ELISA for 15 proteins of interest | KRAS, PIK3CA and BRAF mutation, plasma proteins including angiopeptin 2, interleukin 6, etc | None were predictive of PFS and OS-related benefit of regorafenib |
| CRYSTAL+OPUS (Bokemeyer et al, 2012) | 800/1535 | 1st line, cetuximab+FOLFOX or FOLFIRI vs FOLFOX or FOLFIRI | PCR clamping and melting curve method | KRAS, BRAF mutation | Similar benefit of cetuximab in terms of ORR, PFS and OS in both BRAF-wt and BRAF-mt |
| CO-17 (Karapetis et al, 2014) | 407/572 | 3rd line, cetuximab vs BSC | Nested PCR, IHC | PIK3CA, BRAF mutation and PTEN expression | None were predictive of PFS and OS-related benefit of cetuximab |
| 20100007 (Kim et al, 2018) | 270/377 | 3rd line, panitumumab vs BSC | Sanger sequencing | RAS, BRAF mutation | In BRAF mt (n=20), HR for OS favoured the panitumumab arm (HR 0.39, p=0.1597) and marginal benefit in terms of PFS was shown (HR 0.277, p=0.0002) |

Bev, bevacizumab; BSC, best supportive care; CMS, consensus molecular subtype; CTx, chemotherapy; EGFR, epidermal growth factor receptor; FOLFOX, folic acid, 5-fluorouracil and leucovorin; FOLFIRI, folic acid, 5-fluorouracil and oxaliplatin; IFL, irinotecan, 5-fluorouracil and leucovorin; IHC, immunohistochemical staining; mCRC, metastatic colorectal cancer; mt, mutant; NGS, next-generation sequencing; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; wt, wild-type.

along with chemotherapy (capecitabine or capecitabine plus mitomycin), as opposed to chemotherapy alone. These inconsistent results could be explained by the relatively small number of CMS 1 patients enrolled (less than 20% of the mCRC cases) and different platforms used for gene expression profiling (Almac Xcel array in FIRE-3 and AGITG MAX, and NanoString in CALGB) (table 2). Apart from genetic or transcriptomic profiles, biomarkers such as polymorphisms in VEGF-A or VEGFR-1 or changes in circulating angiogenic factors may be associated with benefits from bevacizumab in mCRC. However, most of these were tested in single-arm studies of bevacizumab, making their predictive impact difficult to assess. A recent study of second-line randomised trials with ramucirumab showed high plasma levels of VEGF-D, a ligand to VEGFR-2, predicted benefits with overall survival (OS) and PFS. These results are contrary to those from CALGB/SWOG 80405, which showed that low VEGFR-D level predicted benefits from bevacizumab-fluorouracil, leucovorin and oxaliplatin (FOLFOX). In the light of such confounding results, the development
and validation of a reliable assay method for the plasma biomarkers is required (table 1).

**Anti-epidermal growth factor receptor antibodies: an arena of diverse biomarkers**

The anti-epidermal growth factor receptor (EGFR) antibodies, cetuximab and panitumumab, have been approved for front-line treatment of mCRC in combination with cytotoxic chemotherapy and later-line treatment as monotherapy or combination therapy. The clinical benefit from anti-EGFR antibodies is restricted to patients with wild-type RAS. Other genetic alterations in the EGFR signalling pathway such as PIK3CA mutation, phosphatase and tensin homolog (PTEN) loss, BRAF mutation and human epidermal growth factor receptor 2 (HER2) amplification have been associated with anti-EGFR resistance in retrospective series or single-arm phase II studies. Although these associations are highly plausible, there has been no statistically significant evidence from randomised trials showing that the magnitude of benefit from anti-EGFR antibodies is significantly jeopardised in such subgroups. This is probably because the incidence of these alterations is so rare that the subgroup analysis lacked sufficient power to prove their association with resistance or because the alterations are less potent than RAS mutations in terms of conferring resistance (table 2).

**RAS** and other genetic alterations that emerge during anti-EGFR treatment detected in tumour tissue as well as ctDNA have recently arisen as markers of acquired resistance. Up to 30%–40% of patients administered with anti-EGFR show RAS mutations in their plasma ctDNA at the time of disease progression. Mutations in the EGFR ectodomain (S492R) also confer resistance to anti-EGFR treatment, although the degree of resistance differs.

### Table 2 Biomarker study from randomised trials comparing anti-VEGF to anti-EGFR therapy for mCRC

| Study—author, year | Number of patients (analysed for biomarker/randomised) | Design | Biomarker analysis platform | Analysed markers | Results |
|--------------------|--------------------------------------------------------|--------|-----------------------------|-----------------|---------|
| CALGB80405 Lenz et al, 2019 | 663/1137 | 1st line, Bev+FOLFOX or FOLFIRI vs Cmab+FOLFOX or FOLFIRI | Nanostring | CMS | Poorer OS (HR 2.34, p<0.001) and PFS (HR 2.28, p<0.001) with Cmab than with Bev in CMS1 (n=104) Better OS (HR 0.62, p=0.0046) with Cmab than with Bev in CMS2 (n=242) |
| Innocenti et al, 2019 | 843/1137 | FoundationOne | 426 genes and 5 microsatellite markers | Promega for MSI | Better OS (HR 0.16, p<0.001) and PFS (HR 0.13, p<0.001) with Bev than with Cmab in MSI-H (n=92) |
| Nixon et al, 2016 | 715/1137 | ELISA | 23 plasma biomarkers | | Low VEGF-D predicted PFS benefit from Bev (HR 1.70) rather than Cmab (HR 0.92) (interaction p=0.0097) Low PiGF predicted PFS benefit from Bev (HR 1.50) rather than Cmab (HR 0.94, interaction p=0.0298) |
| FIRE3 Stintzing et al, 2017 | 313/588 | 1st line, Bev+FOLFIRI vs Cmab+FOLFIRI | Almac Xcel array | CMS | Better PFS (HR 0.63, p=0.031) and OS (HR 0.52, p=0.012) with Cmab than with Bev in CMS4 (n=104) in unadjusted analysis |
| Laurent-Puig et al, 2019 | 340/592 | Taqman assay | miR-31-3p | | Better PFS (HR 0.74, p=0.05), OS (HR 0.61, p<0.01), and objective response with Cmab than with Bev in low miR-31-3p expressers; no difference in high expressers |
| Berger et al, 2017 | 522/586 | PCR-based direct sequencing | SVCT1, SVCT2 and Glut1 gene polymorphism | | SVCT1 CC genotype was associated poorer PFS and OS than any T genotype in Bev arm with KRAS mutation but not in Cmab arm in unadjusted analysis |
| Heinemann et al, 2018 | 373/592 | FoundationOne | 426 genes | | No benefit with Bev in terms of OS (HR 1.17, p=0.82) in MSI-H (n=10); benefit of Cmab was marginally favourable (HR 0.75, p=0.08) in MAPK-wt (n=178); TMB or other markers could not be validated as prognostic or predictive |
| Miller-Philips et al, 2019 | 333/592 | Almac Xcel array | miR-21 | | Better ORR (80.0% vs 57.9%, p<0.005) and OS (HR 0.625, p=0.001) with Cmab than with Bev in low miR-21 subgroup (n=166) |
| Stintzing et al, 2014 | 299/592 | Direct sequencing | AREG SNP rs161511 | | AREG A/G genotype was associated with poorer ORR (38% vs 79%, p=0.02), PFS (HR 3.46, p=0.001) and OS (HR 3.87, p=0.001) compared with G/G genotype in Cmab arm but not in Bev arm |

AREG, amphiregulin; Bev, bevacizumab; Cmab, cetuximab; CMS, consensus molecular subtype; EGFR, epidermal growth factor receptor; FOLFIRI, folinic acid, 5-fluorouracil and oxaliplatin; Glut, glucose transporter; mCRC, metastatic colorectal cancer; MSI, microsatellite instability; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PiGF, placental growth factor; SVCT, sodium-dependent vitamin C transporter; TMB, tumour mutational burden; VEGF, vascular endothelial growth factor.
between cetuximab and panitumumab due to their different binding epitopes. Amplification of receptor tyrosine kinases (HER2 or MET) or BRAF mutations that emerge during anti-EGFR treatment have been suggested to be markers of acquired resistance.

However, the clinical effectiveness of serial monitoring of ctDNA during anti-EGFR treatment has not been well established yet. According to a study that serially measured ctDNA during anti-EGFR treatment, RAS mutations appeared 3–4 months earlier than clinical progression, and the emergence of these mutations was not correlated with PFS. There has been no evidence that early switch of regimen in response to the emergence of ctDNA RAS mutation is more beneficial in terms of OS than conventional switch on clinical progression. However, once clinical progression occurs after anti-EGFR treatment, measurement of ctDNA might be helpful in guiding further treatment. A phase II study in patients previously treated with anti-EGFR proposed ctDNA RAS mutation as a predictors of response to anti-EGFR rechallenge. Knowing the dynamics of emergent RAS mutations after progression could help determine the optimal timing of anti-EGFR antibody rechallenge. A recent study showed that anti-EGFR resistant clones with RAS and EGF mutations at progression after treatment with anti-EGFR antibodies decayed exponentially after anti-EGFR cessation with a cumulative half-life of 4.4 months.

MSI-H has been associated with poor prognosis in patients treated with anti-EGFR antibodies, as compared with bevacizumab. Reduced EGFR ligand expression due to hypermethylation typically seen in MSI-H tumours could explain anti-EGFR resistance; however, not all MSI-H tumours exhibit a hypermethylation phenotype, especially in Asian countries. The precise mechanism of resistance in MSI-H tumours remains unknown.

MSI-H largely overlaps with right primary tumours, which are also adversely associated with anti-EGFR resistance. Subgroup analyses and systematic reviews of randomised trials have consistently revealed a lack of benefit from cetuximab or panitumumab in terms of PFS and OS in right-sided tumours in a front-line setting. Right-sided tumours more frequently harbour biomarkers associated with anti-EGFR resistance (RAS, BRAF, PIK3CA mutations and reduced EGFR ligand expression) than their left-sided counterparts. However, the CALGB study showed that sidedness was negatively associated with poor OS in cetuximab therapy as compared with bevacizumab therapy after adjusting for the aforementioned biomarker profiles. Several studies have shown more favourable tumour shrinkage with anti-EGFR therapy than with bevacizumab for right-sided tumours, suggesting that anti-EGFR antibodies could provide a means of achieving rapid control of tumour volume for certain classes of right-sided tumours.

Transcriptional biomarkers have also been studied in association with cetuximab efficacy (table 2). Upregulation of a specific microRNA, miR-31-3p, plays a significant role in activating RAS signalling and was identified as a potential negative predictor of cetuximab efficacy in the FIRE-3 study. As seen with bevacizumab, the associations between CMS subtype and cetuximab efficacy are inconsistent between studies. A relative benefit of cetuximab as compared with bevacizumab was observed for CMS 4 in the FIRE-3 study but for CMS 2 in the CALGB dataset. The change in CMS has also been associated with acquired resistance; a paired biopsy study revealed that transcriptional change (switch of CMS from 2 to 4) with increased infiltration of cancer-associated fibroblasts was seen in tissues obtained after progression.

From a clinical perspective, front-line anti-EGFR treatment generally produces better objective response rates (ORRs) and increased tumour shrinkage. Thus, this treatment is favoured over bevacizumab, especially for patients with borderline-resectable metastases or with high tumour burdens. However, anti-EGFR treatment usually causes skin toxicity and emotional stress, which could hinder the social lives of patients. Therefore, it is important to select the best-fit candidates for front-line anti-EGFR treatment based on predictive markers such as sidedness, RAS mutation or MSI. Comprehensive tumour profiling such as the PRESSING panel, a platform incorporating NGS, immunohistochemical staining (IHC), in situ hybridisation (ISH) and RNA sequencing, could help in optimising anti-EGFR treatment for mCRC.

**Strategies targeting BRAF-mutant CRC**

The poor prognosis of BRAF V600E mutant CRC has been consistently seen in every clinical trial conducted so far, along with real-world data. Unlike BRAF-mutant melanoma, BRAF mutant CRC does not respond to BRAF inhibitor monotherapy due to parallel EGFR activation by negative downstream feedback. Several clinical trials have tested the strategy of blocking both of upstream (EGFR) and downstream (BRAF) elements of these pathways, which are active in BRAF mutant mCRC (table 3). The SWOG1406 randomised phase II trial showed improved PFS with a combination of vemurafenib (BRAF inhibitor), cetuximab and irinotecan combination (VIC) as compared with just cetuximab and irinotecan. Recently, the BEACON randomised phase III trial showed that the triplet combination of encorafenib (BRAF inhibitor), binimetinib (mitogen-activated protein kinase kinase (MEK) inhibitor) and cetuximab showed improved OS when compared with the control arm (encorafenib+irinotecan-based chemotherapy). In this study, the doublet combination of encorafenib and cetuximab also showed improved OS as compared with the control arm. While this study did not permit cross-over between the arms, 48% of patients in the control arm of the SWOG trial did cross over to receive VIC, resulting in a slight, but statistically insignificant, improvement in OS. Given the high rate of grade 3 or 4 toxicity of the VIC regimen (nausea, diarrhoea and neutropenia in more than 20% of patients), encorafenib-based triplet or doublet combinations, which do not contain cytotoxic agents, appeared to be more feasible options.
for BRAF-mutant patients. The triplet regimen is now being tested for untreated populations in a phase II trial (NCT03693170). Until now, based on the results from subgroup analysis of the TRIBE trial, there has been a consensus that the optimal front-line treatment for BRAF mutant patients might be intensive chemotherapeutic regimens (bevacizumab+5-fluorouracil, oxaliplatin and irinotecan; FOLFOXIRI) to mitigate the aggressive biology. It would be worthwhile to evaluate if the targeted regimen (encorafenib and cetuximab with or without binimetinib) without cytotoxic agents could prove more effective than this intensive combination as a frontline treatment for BRAF V600-mutant mCRC.

Although the V600 mutation is the most common type of BRAF mutation, non-V600 BRAF mutations are being more frequently detected with NGS tests becoming more widely available. Non-V600 mutations account for 20%–40% of all BRAF mutations in mCRC and represent different clinical characteristics from those of V600 mutants. These differences manifest as fewer female patients, lower histological grades and greater incidence of left-sideness in the non-V600 mutants as compared with V600 mutants. Based on the degrees of RAS-dimer and RAF-dimer dependency in the signalling pathway, BRAF mutations are categorised as class 1 (V600 mutants: RAS-independent, dimer-independent and kinase-active), class 2 (RAS-independent, dimer-dependent and kinase-active) and class 3 (RAS-dependent, dimer-dependent and kinase-inactive). BRAF inhibitors show limited activity in class 2 and 3 mutants, which exhibit RAF dimer-dependent signalling. Class 3 BRAF mutations, which comprise more than half of non-V600 BRAF mutants, frequently overlap with RAS mutations. However, in the case of class 3 BRAF mutants with wild-type RAS, inhibiting the RAS signal with anti-EGFR antibodies could be a reasonable option; moreover, anti-EGFR inhibitors when combined with MEK inhibitor can prevent feedback activation by BRAF inhibition and have been proposed as a more rational approach. Class 2 BRAF mutants are difficult to target due to RAS-independent kinase activity, although treatment options such as combinations of anti-EGFR, MEK and/or ERK inhibitors would be worth further exploration. For non-V600 BRAF mutant mCRC, the triplet regimen from the BEACON trial is currently being tested in a phase II trial (UMIN000031857).

**HER2 blockades in CRC**

HER2 amplification, observed in 2%–4% of mCRC cases, shows a predilection for the left colon or rectum and is mainly enriched in RAS and BRAF wild-type cancer; however, it has also been associated with anti-EGFR resistance. Unlike breast cancer, anti-HER2 antibody (trastuzumab) monotherapy has not been successful in treating HER2-amplified mCRC. This is likely due to delayed EGFR and HER3 activation following trastuzumab monotherapy may cause intrinsic resistance. Dual blockade targeting HER2 and EGFR/HER3 is therefore required for this disease subset.

Several trials have shown the clinical activity of a combination strategy for HER2 blockade (table 4). The HERACLES investigators defined certain CRC-specific criteria for IHC staining in HER2, which were concordant with ISH parameters, and screened more than 900 KRAS wild-type patients, of which 5% were HER2 positive. Trastuzumab and lapatinib showed promising activity in the heavily treated patients, and correlative biomarker analysis revealed the HER2 copy number in the tissue and ctDNA predicted the response to the treatment. The results of the MyPathway trial demonstrated the effectiveness of the combining pertuzumab with trastuzumab for this population, with a profound difference in outcome based on KRAS mutation status (table 4). DS-8201a, a novel HER2-targeted antibody-drug conjugate with trastuzumab and topoisomerase I inhibitor (deruxtecan) payload, also

| Study (author, year) | Phase | N | Eligibility | Treatment | Results |
|---------------------|-------|---|-------------|-----------|---------|
| BEACON (Kopetz et al, 2019) | III | 665 | PD after 1 or 2 prior treatments | Triplet: encorafenib+binimetinib+Cmab<br>Doublet: encorafenib+Cmab<br>Control: Cmab+irinotecan or Cmab+FOLFIRI | Triplet vs doublet vs control: OS 9.0m vs 8.4m vs 5.4m (p=0.001)<br>PFS 4.3m vs 4.2m vs 1.5m (p=0.0001)<br>ORR 26% vs 20% vs 2% (p<0.001) |
| SWOG S1406 (Kopetz et al, 2017) | II | 106 | PD after 1 or 2 prior treatments | Vemurafenib+cetuximab+irinotecan vs cetuximab+irinotecan | PFS 4.3m vs 2.0m (p=0.001)<br>OS 9.6m vs 5.9m (p=0.19) |
| NCT01750918 (Corcoran et al, 2015) | II | 43 | Any line | Dabrafenib+trametinib | ORR 12%, PFS 3.5m |
| NCT01750918 (Corcoran et al, 2018) | I | 20 | Any line | Pmab+trametinib | ORR 0%, PFS 2.6m |
| NCT01750918 | I | 91 | ≥1 prior treatment | Dabrafenib+Pmab+trametinib | ORR 21%, PFS 4.2m |
| NCT01750918 | I | 20 | | Dabrafenib+Pmab | ORR 10%, PFS 3.5m |
| NCT01750918 | I | 26 | | Encorafenib+Cmab+alpelisib | ORR 17.9%, PFS 4.2m |
| NCT01750918 | I | 26 | | Encorafenib+Cmab | ORR 19.2%, PFS 3.7m |

Cmab, cetuximab; FOLFIRI, folinic acid, 5-fluorouracil and irinotecan; m, months; ORR, overall response rate; OS, overall survival; PD, progressive disease; PFS, progression-free survival; Pmab, panitumumab.
demonstrated clinical activity in HER2+ mCRC in the dosing expansion cohort of a phase I trial, thus warranting the currently ongoing phase II trial. Recently, a study from Japan showed that ctDNA could be used for negative selection of candidates for this combination; patients with ctDNA alterations of RAS, BRAF, PIK3CA and HER2 did not benefit from the dual HER2 blockade. Another dual combination comprising inhibitors of HER2, trastuzumab and tucatinib showed significant activity, with an ORR of 55% and median PFS of 6.2 months in 22 patients, while pertuzumab and trastuzumab-emtansine (T-DM1) produced an ORR of 10%, which did not meet the primary endpoint. With advancements in efficient HER2 blockades, including antibody–drug conjugates, bispecific antibodies, small molecule inhibitors or combinations with immunotherapy, more innovative therapeutics could emerge in this field.

**RAS inhibitors: targeting the ‘undruggable’ genetic alterations**

RAS mutations are notoriously undruggable targets due to their molecular structures with deeply seated hydrophobic pockets which are difficult to target using small molecules. Recent discoveries have allowed for the development of small-molecule inhibitors that selectively bind to a newly discovered allosteric regulatory site of the G12C mutant form of KRAS is underway. Preliminary data of a phase I study of AMG 510, the first-in-class KRAS G12C inhibitor, showed that approximately half of patients with non-small cell lung cancer achieved a partial response; however, an objective response was rare in the case of CRC. Because of the suboptimal activity in KRAS G12C mutant mCRC, which is a rare occurrence comprising approximately 4% of CRC cases, drug development in this field might be more challenging than expected. Recently, a preclinical study has suggested synergism between cetuximab and AMG 510 in KRAS G12C mutated CRC, implying that the combination could be explored as an alternative approach. Meanwhile, one can explore the upcoming results of different KRAS G12C inhibitors, as well as mutant-specific agents targeting more common variants such as KRAS G12D or G12V.

**Treating patients with rare genetic alterations**

Other than BRAF or HER2, even rarer genetic alterations in mCRC are now being considered as actionable targets. ALK, ROS1, NTRK, RET or FGFR2,3 fusions are rarely detected in mCRC, occurring in less than 1% of cases. For ALK, ROS1 and NTRK fusions, entrectinib may be useful as a tissue-agnostic therapeutic approach, although the response duration of patients with mCRC generally seems to be limited as compared with those in other disease subsets such as lung cancer or sarcoma. Larotrectinib, a selective TRK inhibitor, was also seen to be active in patients with NTRK fusion-positive solid tumours, 7% (4/55) of whom had mCRC. Therefore, patients with rare fusions can obtain clinical benefit from targeted agents, although the challenge lies in identifying these patients. DNA-level sequencing panels have limitations, especially for large genes such as NTRK2 or NTRK3, for which RNA-based sequencing assays are usually needed.

| Study (author/year) | Phase | N | Eligibility | Treatment | Results |
|---------------------|-------|---|-------------|-----------|---------|
| HERACLES (Sartore-Bianchi et al, 2016) | II | 27 | KRAS wt, progression after all standard treatments, HER2+ by HERACLES criteria | Trastuzumab+lapatinib | ORR 30% (95% CI 14 to 50) PFS 21 weeks (95% CI 16 to 32) |
| Phase 1 dose expansion cohort of DS-8201a (Yoshino et al, 2018) | I | 19 | HER2 IHC ≥1+ or HER2 amplified | DS-8201a | ORR 15.9% (3/19) DCR 82.4% (16/19) PFS 3.9 m (95% CI 2.1 to 8.3) |
| MyPathway (Meric-Bernstam et al, 2019) | II | 57 | ≥7 prior treatments, HER2+ by ISH, NGS or IHC | Trastuzumab+pertuzumab | ORR 32% (95% CI 20 to 45), PFS 2.9 m (95% CI 1.4 to 5.3) ORR 40%, PFS 5.3 m in KRAS wt (n=43) ORR 8%, PFS 1.4 m in KRAS mt (n=13) |
| TRIUMPH (Nakamura et al, 2019) | II | 18 | Tissue and/or ctDNA (Guardant360) confirmed RAS-wt and HER2-amplified mCRC | Trastuzumab+pertuzumab | Tissue-positive: ORR 35% (95% CI 14 to 62), PFS 4.0 m (95% CI 1.4 to 5.6) ctDNA-positive: ORR 33% (95% CI 12 to 62), PFS 4.0 m (95% CI 1.3 to 5.6) |
| MOUNTAINEER (Strickler et al, 2019) | II | 22 | HER2+ by NGS, ISH or IHC prior 5-FU, OXA, IRI, anti-VEGF | Trastuzumab+tucatinib | ORR 55% PFS 6.2 m (95% CI 3.5 to NE) |
| HERACLES-B (Sartore-Bianchi et al, 2019) | II | 30 | RAS/BRAF wt, HER2+ by HERACLES criteria, progression after 5-FU, OXA, IRI, anti-EGFR | Trastuzumab+T-DM1 | ORR 10% (95% CI 0 to 28) PFS 4.8 m (95% CI 3.6 to 5.8) |

ctDNA, circulating tumour DNA; EGFR, epidermal growth factor receptor; 5-FU, 5-fluouracil; IHC, immunohistochemical staining; IRI, irinotecan; ISH, in situ hybridisation; m, months; mCRC, metastatic colorectal cancer; mt, mutant; NE, not estimated; NGS, next-generation sequencing; ORR, overall response rate; OXA, oxaliplatin; PFS, progression-free survival; VEGF, vascular endothelial growth factor; wt, wild-type.
for reliable detection of fusions. However, it is not feasible in daily practice for all patients with mCRC to undergo RNA sequencing to detect rare genetic events. Although the clinical characteristics of patients with actionable fusions have been identified (elderly and female patients with right-sided and MSI-H tumours), it is uncertain whether limiting fusion testing of those patients would be an efficient method of screening. IHC could be a feasible alternative for fusion detection; these methods for detection of ALK and NTRK fusions showed varying rates of concordance with fluorescent ISH (FISH) and RNA-based sequencing in mCRC. However, we currently lack sufficient data on IHC for ROS1 or other fusions.

Ubiquitin ligase ring finger protein 43 (RNF43) is a negative regulator of the Wnt pathway. Somatic mutations in RNF43 occur in 6%–18% of CRC cases. Truncating mutations of RNF43 appear mutually exclusively with APC mutations, which is also associated with Wnt pathway activation. Fusions in RSPO2 or RSPO3 (secreted agonists of the Wnt-β-catenin pathway) are detected in approximately 10% of CRC cases and also avert APC mutations. These genetic alterations mainly overlap with MSI-H, which is a target for immune checkpoint inhibitors (ICIs). They may also be targeted by inhibiting porcupine, a protein involved in Wnt secretion. A high-throughput drug screening study using organoids showed that a colorectal tumour organoid with an RNF43 mutation was sensitive to IWP2, a small molecule porcupine inhibitor. Patient-derived xenografts of gastrointestinal cancer harbouring an RSPO2 fusion were also effectively treated by the porcupine inhibitor CGX1321. A recent phase I study of the first-in-class porcupine inhibitor WNT974 showed tumour regression in a case of appendiceal cancer with an RNF43 mutation. WNT974 is also being tested in patients with mCRC with BRAFV600E and RNF43 mutations or RSPO fusions, in combination with BRAF inhibitor and anti-EGFR to mitigate acquired resistance through the Wnt-β-catenin pathway, in a phase II study (NCT02278133) and in combination with the ICI PD001 (NCT01351103).

Immunotherapy for CRC: for and beyond MSI-H

MSI-H has been established as a reliable biomarker that predicts benefit from ICI in mCRC, as well as other types of cancers. Although MSI-H tumours comprises only 3%–5% of mCRC cases, they show high tumour mutational burden (TMB), high programmed-death ligand-1 (PD-L1) expression and high neoantigen load, making the tumour cells easily identifiable by immune system and sensitive to PD-1 or PD-L1 antagonists. Multicentre clinical trials with anti-PD-1 antibodies such as pembrolizumab and nivolumab have demonstrated favourable ORR (around 30%) and durable survival outcomes, with PFS at 12 months of 30%–50% and OS at 12 months of 70%–80% in pretreated patients with MSI-H mCRC. Phase III trials of these agents as compared with standard front-line regimens have been conducted and the results are awaited within the next few years: KEYNOTE 177 for pembrolizumab (NCT02563002) and CHECKMATE 8HW for nivolumab (NCT04008030). A combination of nivolumab and ipilimumab, an anti-cytotoxic T-lymphocyte–associated antigen (CTLA)-4 antibody, was also shown to be active in a pretreated population with MSI-H mCRC (ORR 55%, PFS at 12 months 71%) as well as untreated patients (ORR 60%, PFS at 12 months 77%). The activity of avelumab, an anti-PD-L1 antibody, is under investigation for MSI-H mCRC by our group, and the preliminary results have been promising in terms of ORR (30% in mCRC with MSI-H as defined by Bethesda panel or NGS). Recent translational studies have focused on the molecular heterogeneity within MSI-H tumours and its impact on clinical benefits from ICI treatment; higher TMB, especially insertion-deletion mutational load, has been known to be associated with the extent of response.

Despite all the aforementioned developments, researchers are still struggling to identify valid immunotherapeutic options for microsatellite-stable (MSS) CRC, which shows no evidence of objective response to ICIs. A combination of MEK inhibitor with anti-PD-1 antibodies appeared to be active in preclinical studies and a phase I trial; however, the IMblaze370 study, a phase III trial of atezolizumab and cabitinib, showed no improvement in OS or PFS when compared with regorafenib. Another randomised trial of ICIs for mCRC, the MODUL study, compared bevacizumab, fluoropyrimidine-atezolizumab with bevacizumab and fluoropyrimidine. The study also failed to show benefits in terms of PFS, the primary endpoint, as well as overall response rate, disease control rate and duration of response. Although the TMZ of MSS mCRC is much lower than that of MSI-H mCRC, the cause of intrinsic immune resistance of MSS mCRC cannot solely be explained by TMZ because other types of cancers with similar TMZ as MSS mCRC do respond to ICIs. Hence, the mechanism of de novo immune resistance of MSS CRC remains incompletely addressed. Recent translational studies have indicated that activated Wnt/β-catenin signalling and transforming growth factor beta (TGF-β) signalling may cause T-cell exclusion and immune evasion in mCRC. In addition, a preclinical study showed that oncogenic KRAS mutations induce immunosuppression by downregulating interferon regulatory factor 2 (IRF2), resulting in activation of the CXCL3–CXCR2 axis and recruitment of myeloid-derived suppressive cells in CRC. This also suggests the possibility of combining ICI with CXCR2 inhibitor as a viable option to overcome the immunosuppressive microenvironment.

Several clinical trials have suggested that combination strategies with ICI could work as therapeutics for MSS mCRC. The combination of ICI, tremelimumab (anti-CTLA-4 antibody) and durvalumab (anti-PD-L1) showed a slight improvement in OS (6.6 vs 4.1 months, stratified HR 0.72, 95% CI 0.54 to 0.97; p=0.07), but not PFS, in heavily treated patients with mCRC, mostly with MSS
type. In addition, a phase I trial of regorafenib and nivolumab showed remarkable objective response (33%, 8/24) in patients with MSS mCRC, which spurred a phase III trial in refractory settings. Other early-phase studies of combination strategies, which showed anecdotal objective responses in MSS mCRC, are also intriguing and worth following for updated results, including BB1-608, a STAT3 inhibitor, combined with pembrolizumab as well as monalizumab, an anti-NKG2A (checkpoint of NK cell) antibody, with durvalumab+cetuximab.

CONCLUSION

Even in the current era of precision medicine, there remain significant unmet needs for patients with mCRC. Most of the known actionable targets (BRAF, HER2, ALK, ROSI and NTRK, as well as MSI-H for immunotherapy) are rarely present, and prevalent oncogenic genetic alterations such as APC, TP53 and RAS have been generally undruggable thus far. CMS has emerged as a prognostic or predictive marker of targeted therapy; however, substantial work is required for more robust classification of subtypes across different platforms and diverse clinical settings. Ongoing efforts to share and integrate clinical and genomic data could help in the discovery and validation of new actionable targets. Combinations of target blockades and ICIs could provide potential therapeutic opportunities for mCRC cases lacking drug-target blockades and ICIs could provide potential therapeutics. The first author drafted the manuscript under the guidance by the corresponding author, and the all authors revised and agreed on the final version of manuscript.

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