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REVIEW ARTICLE

The Evolving Biomarker Landscape for Treatment Selection in Metastatic Colorectal Cancer

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Abstract

The approval of targeted therapies for metastatic colorectal cancer (mCRC) has led to important improvements in patient outcomes. However, it is still necessary to increase individualisation of treatments based on tumour genetic profiles to optimise efficacy, while minimising toxicity. As such, there is currently great focus on the discovery and validation of further biomarkers in mCRC, with many new potential prognostic and predictive markers being identified alongside developments in patient molecular profiling technologies. Here, we review data for validated and emerging biomarkers impacting treatment strategies in mCRC. We completed a structured literature search of the PubMed database to identify relevant publications, limiting for English-language publications published between 1 January 2014 and 11 July 2018. In addition, we performed a manual search of the key general oncology and CRC-focused congresses to identify abstracts reporting emerging mCRC biomarker data, and of ClinicalTrials.gov to identify ongoing clinical trials investigating emerging biomarkers in mCRC and/or molecular-guided clinical trials. There is solid evidence supporting the use of *BRAF* status as a prognostic biomarker and *DYPD*, *UGT1A1*, *RAS*, and microsatellite instability as predictive biomarkers in mCRC. There are a number of emerging biomarkers that may prove to be clinically relevant in the future to have prognostic (*HPP1* methylation), predictive (*HER3*, microRNAs, anti-angiogenic markers, and CRC intrinsic subtypes), or both prognostic and predictive values (*HER2*, CpG island methylator phenotype, tumour mutational load, gene fusions, and consensus molecular subtypes). As such, new biomarker-led treatment strategies in addition to anti-epidermal growth factor receptor and anti-angiogenetic treatments are being explored. Biomarkers that are not recommended to be tested in clinical practice or are unlikely to be imminently clinically relevant for mCRC include thymidylate transferase, ERCC1, *PIK3CA*, and *PTEN*. We highlight the clinical utility of existing and emerging biomarkers in mCRC and provide recommended treatment strategies according to the biomarker status. An update on ongoing molecular-guided clinical trials is also provided.

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Key Points for Decision Makers

With the approval of therapies that specifically target the molecular differences between normal cells and cancer cells, there is a strong need to ensure that the most beneficial therapeutic strategies are adopted for each patient.

Therapies can be targeted appropriately by assessing the presence of biomarkers.

The biomarker landscape in metastatic colorectal cancer is evolving and we provide guidance on which biomarkers currently are (*DPYD*, *UGT1A1*, *RAS,* microsatellite instability), and may become (*BRAF, HER2*, consensus molecular subtypes, CRC intrinsic subtypes, *EGFR, HER3*, microRNA, anti-angiogenic markers, tumour mutational load, gene fusions, CpG island methylator phenotype), most relevant for clinical practice.

We recommend treatment strategies according to the presence or absence of biomarkers including *RAS*, MSI, *BRAF*, and *HER2* and provide an update on ongoing molecular-guided clinical trials, which will further individualise therapy for patients with mCRC.

1 Introduction

Colorectal cancer (CRC) is one of the most diagnosed cancers worldwide, with 1.84 million estimated new cases in 2018 [1]. Fluorouracil (5-FU) was the historic standard of care for patients with CRC, but the treatment landscape has evolved rapidly in the metastatic setting following the approval of several targeted therapies, leading to improvements in tumour response rates and patient survival [2]. Despite the multitude of treatments available, outcomes and toxicity with each regimen can vary markedly from patient to patient [3]. Therefore, there is a strong need to identify disease and host biomarkers that will ensure the most beneficial therapeutic strategy is adopted for each patient.

Although primary tumour location [right-sided (located in the caecum to transverse colon) or left-sided (located from the splenic flexure to rectum)] has been identified as a surrogate marker for tumour biology [4–6], more accurate knowledge of a patient's tumour profile is needed to better personalise treatment. Indeed, in recent years there has been a great focus on the development of biomarkers in metastatic CRC (mCRC) [6–9], all with the aim of improving outcomes for patients, including avoiding missed treatment opportunities or unnecessary toxicity. These have included new diagnostic biomarkers (for disease detection and cancer staging or risk stratification), new predictive biomarkers (to predict patient response to therapy) and new prognostic biomarkers (to assess how the disease is likely to evolve). Based on these efforts, testing for some biomarkers is now standard practice in the mCRC setting. Newer technologies such as next-generation sequencing (NGS) and tumour panels have highlighted many more potential predictive and prognostic markers. While these techniques can provide a wealth of information, their application in clinical practice is not always straightforward [10, 11]. There is a clear need for evidence-based recommendations to guide the use of validated and emerging biomarkers in clinical practice. Here we review the clinical utility of existing and emerging biomarkers that are being used or investigated to support treatment decisions for patients with mCRC, including those who develop acquired resistance to treatment.

2 Methods

We completed a structured literature search to identify relevant publications. The PubMed database (www.ncbi.nlm.nih.gov/pubmed/) was searched using the following terms and restrictions: ("metastatic colorectal cancer"[Title/Abstract] OR "mCRC"[Title/Abstract]) AND ("biomarkers"[Title/Abstract] OR "molecular"[Title/Abstract] OR "molecular guided"[Title/Abstract] OR "tumor board"[Title/Abstract]), limiting for English-language publications (specifically of clinical trials, meta-analyses, observational studies, comparative studies, clinical studies, systematic reviews, multicentre studies, or case reports) published between 1 January 2014 and 11 July 2018. The search produced 519 hits. The titles and abstracts of these publications were reviewed and the full-text versions of manuscripts reporting emerging mCRC biomarker data were retrieved and reviewed in detail. In addition, we performed a manual search of the key general oncology and CRC-focused congresses to identify abstracts reporting emerging mCRC biomarker data (published between 1 January 2015 and 11 July 2018). We also performed a manual search of ClinicalTrials.gov to identify ongoing clinical trials investigating emerging biomarkers in mCRC and/or molecular-guided clinical trials.

3 Biomarkers and Chemotherapy in mCRC

Neoadjuvant and adjuvant chemotherapy with fluoropyrimidine-based regimens are beneficial for many patients with mCRC, and several markers of chemotherapy sensitivity or toxicity have been proposed. Dihydropyrimidine dehydrogenase (DPD) is an enzyme encoded by the *DPYD* gene that catalyses the inactivation of some fluoropyrimidines, and its deficiency is associated with increased chemotherapy-related toxicity [12–14]. *DPYD* allelic variants that are associated with severe toxicity include *DPYD*2A* and A2846T [12,

15–17]. Other variants have been identified but their clinical relevance remains to be confirmed [15]. The European Society for Medical Oncology (ESMO) guidelines do not recommend systematic DPD testing before 5-FU or capecitabine administration (Table 1), although testing remains a good option, with some groups calling for *DPYD* genotype- and/or phenotype-guided individualised dosing to be a new standard of care [3, 17]. Indeed, DPD testing is standard practice in some European countries, including France [18]. Given that fluoropyrimidine treatment can result in severe toxicity in up to 39% of patients [17], DPD testing, which is feasible in routine clinical practice, may be of value and will probably be extended to other European countries in forthcoming years. Other potential markers of toxicity or response associated with fluoropyrimidines are yet to be validated, including genetic variations in the thymidylate synthase gene and microRNA (miRNA)-143 [3, 19]. Polymorphisms in the gene encoding UDP glucuronosyltransferase 1 family, polypeptide A1 (*UGT1A1*) have also been linked with tolerance to chemotherapy [20]. While recent data from the PETACC-3 trial confirmed an association between *UGT1A1*28* genotype and chemotherapy-dependent toxicity, other clinical parameters (including sex, age and performance status) were found to be stronger predictors of toxicity risk [21]. Further to this, a meta-analysis revealed an association between *UGT1A1*6* polymorphisms and irinotecan-induced toxicity in Asian patients [22]. Patients heterozygous for *UGT1A1*6* were found to be at increased risk for severe neutropenia, while patients who were homozygous for *UGT1A1*6* were found to be at even higher risk for neutropenia and were also more likely to suffer from severe diarrhoea [22]. *UGT1A1* genotyping/phenotyping is not recommended as a predictive biomarker in everyday practice, but remains an option and should be conducted when UGT1A1 deficiency is suspected, as indicated by low conjugated bilirubin, and when administration of > 180 mg/m^2 irinotecan is planned (Table 1) [3, 23]. The frequency of *UGT1A1*6* is higher, while the frequency of *UGT1A1*28* genotype is lower, in Asian versus Caucasian patients [23]. Therefore, the Pan-Asian-adapted ESMO consensus guidelines for the management of patients with mCRC also recommend that a lower irinotecan threshold dose for genotyping may be used depending on the prevalence of *UGT1A1* polymorphisms per country [23].

A number of studies have indicated that the excision repair cross-complementation group 1 (ERCC1) protein is a possible prognostic biomarker in platinum-based treatment of metastatic cancers [7, 24–26]. However, survival outcomes did not significantly differ in patients with high versus low baseline ERCC1 levels who received bevacizumab plus mFOLFOX6 (leucovorin [folinic acid], 5-FU, oxaliplatin) or FOLFIRI (leucovorin, 5-FU, irinotecan) in the MAVERICC trial, the first prospective study to investigate ERCC1 as a potential biomarker for oxaliplatin-containing regimens in patients with untreated mCRC [27, 28]. It is not recommended as a biomarker in clinical practice (Table 1) [3].

4 Biomarkers and Anti-EGFR Therapy in mCRC

The development and progression of CRC is influenced by epidermal growth factor receptor (EGFR) and its downstream signalling pathways (Fig. 1) [29]. Therefore, investigation of predictive and prognostic biomarkers historically focused on EGFR expression and subsequently on alterations in the RAS/BRAF/MEK/MAPK and PI3K/PTEN/AKT pathways. Data from clinical trials demonstrate that across all lines of therapy *RAS* mutations predict a lack of response to monoclonal antibodies directed against EGFR (panitumumab/cetuximab) and potentially a detrimental effect of such therapies when combined with oxaliplatin-based chemotherapy [30–34]. Effective first- and second-line therapies are therefore needed for patients with *RAS*-mutated mCRC. Chemotherapy plus bevacizumab is a standard first-line therapy for these patients (Fig. 2) [3, 35], but has limitations. Specifically, *RAS* mutations may be associated with lesser benefit from chemotherapy plus bevacizumab compared with *RAS* wild type (WT) mCRC [36–38], although the recent JACCRO CC-11 trial suggests that first-line mFOLFOXIRI (leucovorin, 5-FU, oxaliplatin, irinotecan) plus bevacizumab is effective for patients with *RAS*-mutated mCRC [39]. Treatment with aflibercept or ramucirumab (in combination with FOLFIRI) may be efficacious as second-line therapies for patients with *RAS*-mutated mCRC [3, 40]. Inhibitors of some mutant forms of RAS, such as KRAS G12C, are now entering clinical trials [41, 42].

Testing for *RAS* mutational status is recommended for all patients at the time of mCRC diagnosis (Table 1) [3, 7]. Initially only mutations in exon 2 of *KRAS* (which lead to constitutive activation of EGFR) were routinely tested. A prospective–retrospective biomarker analysis of the Phase III PRIME study reported that the presence of additional *RAS* mutations (*KRAS* exons 3/4 and *NRAS* exon 2/3/4) also predict a lack of response to panitumumab plus FOLFOX [31]. This observation was subsequently confirmed by retrospective and prospective analyses of other trials of anti-EGFR therapies [43–48]. These mutations are now tested for in extended *RAS* analysis [49]. As around 20% of *KRAS* exon 2 WT tumours harbour a different *RAS* mutation, extended *RAS* testing has significantly impacted clinical outcomes [34]. An NGS-based extended *RAS* panel has recently been clinically validated using formalin-fixed paraffin-embedded mCRC tumour samples [10]. Of note however, a recently reported prospective study of more than 400 patients demonstrated that testing circulating tumour DNA (ctDNA) for

Table 1 Summary of recommendations for biomarker testing according to consensus guidelines for the management of patients with mCRC from ESMO and the American Society for Clinical Pathology, College of American Pathologists, Association for Molecular Pathology, and American Society of Clinical Oncology [3, 7]

Biomarker	Recommendation
DPD (DPYD)	Testing before 5-FU or capecitabine administration remains an option but is not routinely recommended in all European countries[a]
TS	Testing not recommended in clinical practice
UGT1A1	*UGT1A1* phenotyping remains an option and should be carried out in patients with a suspicion of *UGT1A1* deficiency as reflected by low conjugated bilirubin and in patients where an irinotecan dose of > 180 mg/m^2 per administration is planned
ERCC1	Testing not recommended for treatment decisions, could be included prospectively in clinical trials
RAS (KRAS, NRAS)	Mandatory test before treatment with anti-EGFR-targeting antibodies cetuximab or panitumumab
BRAF	Test alongside *RAS* for prognostic role and/or for selection in clinical trials
EGFR	Evaluation of *EGFR* amplification, gene copy number and *EGFR* ectodomain mutations is not recommended for routine patient management outside of a clinical trial setting
PIK3CA	Testing not recommended for routine clinical practice outside of a clinical trial setting
PTEN	Testing not recommended for routine clinical practice outside of a clinical trial setting
MSI	Test for predictive value for the use of immune checkpoint inhibitors (pembrolizumab, nivolumab ± ipilimumab)

5-FU fluorouracil, *BRAF* B-rapidly accelerated fibrosarcoma, *DPD* dihydropyrimidine dehydrogenase, *DPYD* DPD gene, *EGFR* epidermal growth factor receptor, *ERCC1* excision repair cross-complementation group 1, *ESMO* European Society for Medical Oncology, *KRAS* Kirsten rat sarcoma viral oncogene, *mCRC* metastatic colorectal cancer, *MSI* microsatellite instability, *NRAS* neuroblastoma RAS, *PIK3CA* phosphatidylinositol 3-kinase catalytic subunit alpha, *PTEN* phosphatase and tensin homolog, *RAS* rat sarcoma, *TS* thymidylate transferase, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1

[a]Testing is recommended in some European countries [18]

RAS correlated well with tissue assessment, with increased accuracy for patients with liver metastases [50]. While still not recommended by current guidelines [3, 7], ctDNA testing could therefore potentially replace tissue assessment as routine practice in these patients.

Clinical trial data suggest that the mutated *BRAF* V600E is a negative prognostic marker for patients with mCRC and may predict resistance to EGFR-antibody therapy, especially in heavily pre-treated patients [51–53]; the predictive value of *BRAF* V600E mutations in earlier lines of therapy is uncertain [54, 55]. A recent meta-analysis of randomised controlled trials suggested there was insufficient evidence to demonstrate that *BRAF* V600E mutations are a negative predictive marker of response to EGFR inhibitors [56], while a second meta-analysis demonstrated that anti-EGFR treatment did not increase progression-free survival (PFS) or overall response rate (ORR) in patients with *BRAF*-mutated mCRC [57]. However, the outcomes of these meta-analyses are debatable as the analyses included studies with different patient populations, lines of therapy, control arms and anti-EGFR treatment options. More recently, analysis of the VOLFI trial found an impressively increased response rate in *BRAF*-mutated patients receiving first-line panitumumab plus a triplet chemotherapy regimen versus triplet chemotherapy alone (86% vs 22%), although PFS was comparable in the two treatment arms [58]. Of note, the sample size was small ($n = 16$) and a cautious interpretation is warranted [58]. On balance, accumulating evidence suggests that anti-EGFR therapy may be of interest in patients with *BRAF*-mutated mCRC, if used in earlier rather than later lines of therapy, but this is not currently a first-choice therapy in this setting (Fig. 2). As demonstrated in a small subgroup analysis of the TRIBE study [36], and other patient cohorts [59, 60], FOLFOXIRI plus bevacizumab may also be a beneficial first-line treatment for these patients and is recommended by ESMO guidelines for patients with *BRAF*-mutated mCRC (Fig. 2) [3]. Other vascular endothelial growth factor (VEGF) targeting agents may also be efficacious in this patient population. Tumour samples were obtained for 482/1226 (39%) patients randomised in the VELOUR clinical trial, which demonstrated that aflibercept in combination with FOLFIRI is a beneficial second-line treatment for mCRC [61]. Patients with *BRAF*-mutated mCRC ($n = 36$) had a larger benefit from the addition of aflibercept versus placebo to FOLFIRI (median OS 10.3 vs 5.5 months) compared with patients with WT *BRAF* (13.0 vs 12.4 months) [61]. However, the difference was not significant [HR 0.49 (95% CI 0.22–1.09), $p = 0.08$], possibly due to the small series of patients [61]. Similar results were reported in a biomarker analysis of the RAISE trial, where the addition of ramucirumab to FOLFIRI provided a non-significant benefit in *BRAF*-mutated tumours [62].

Inhibition of BRAF V600E has been shown to cause rapid feedback activation of EGFR, which supports continued tumour proliferation [63]. As such, inhibition of EGFR has been shown to be strongly synergistic with BRAF V600E

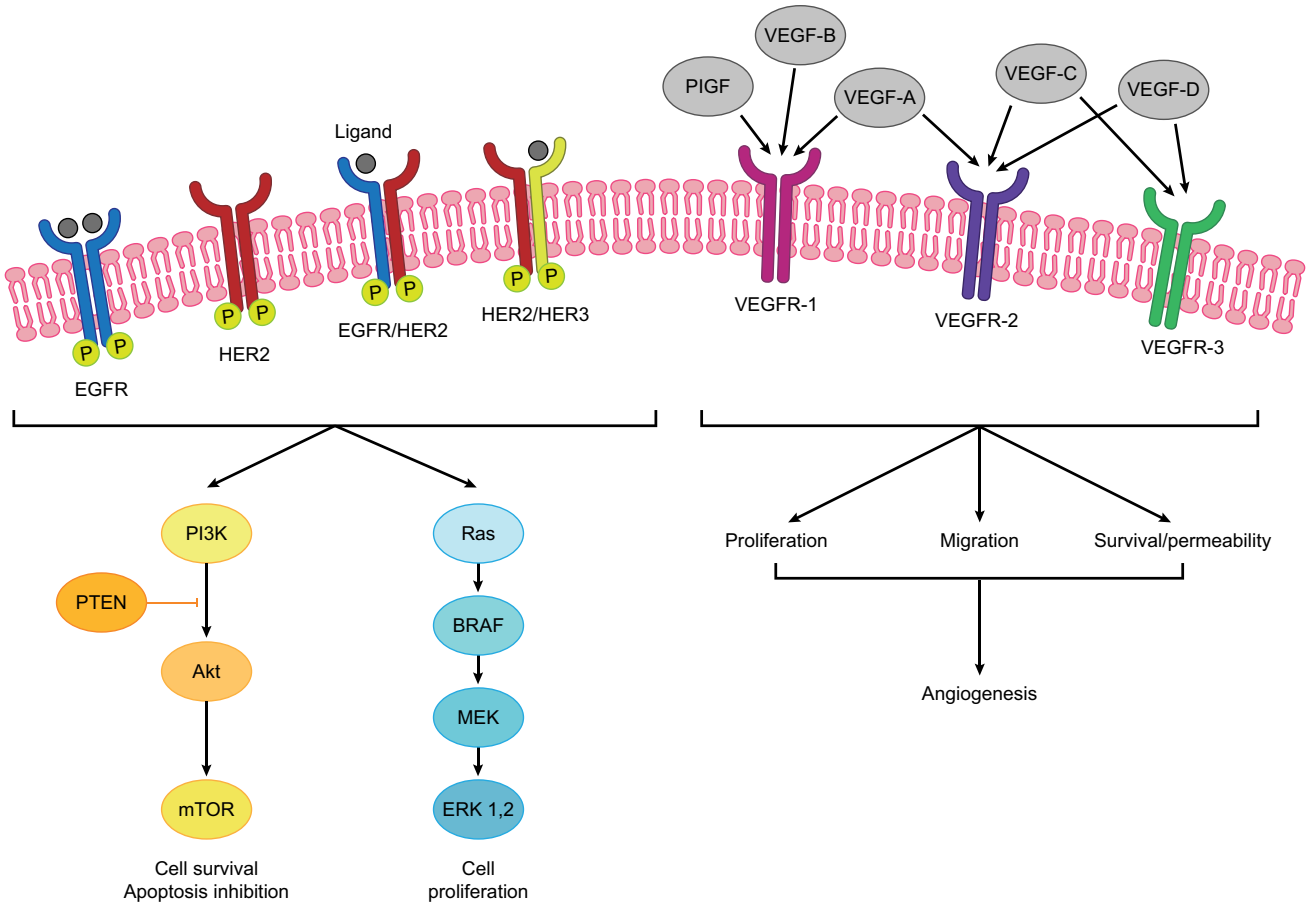

Fig. 1 Overview of the main EGFR and VEGF angiogenic signalling cascades. Upon EGFR dimerisation and autophosphorylation, the RAS/BRAF/MEK and PI3K/PTEN/AKT pathways are induced (adapted from [29] under the Creative Commons Attribution License CC BY-NC 3.0 [https://creativecommons.org/licenses/by-nc/3.0/]). Ligand binding to VEGFR-1, VEGFR-2, and VEGFR-3 activates a number of processes that drive angiogenesis. *AKT* AKR mouse thymoma, *BRAF* B-rapidly accelerated fibrosarcoma, *EGFR* epidermal growth factor receptor, *ERK* extracellular receptor kinase, *HER* human epidermal growth factor, *MEK* mitogen-activated protein kinase, *mTOR* mammalian target of rapamycin, *P* phosphorylation, *PI3K* phosphatidylinositol 3-kinase, *PIGF* phosphatidylinositol-glycan biosynthesis class F, *PTEN* phosphatase and tensin homolog, *RAS* rat sarcoma, *VEGF* vascular endothelial growth factor, *VEGFR* VEGF receptor

inhibition in CRC (Fig. 2) [63]. An open-label Phase I/II study demonstrated that the BRAF inhibitor dabrafenib plus trametinib (a MEK inhibitor) had activity in patients with *BRAF* V600E mutation-positive mCRC, and patients receiving triple therapy (dabrafenib, trametinib and panitumumab) had a numerically improved ORR (21%) compared with those receiving panitumumab plus either dabrafenib (10%) or trametinib (0%) and had a longer PFS (4.2 vs 3.5 or 2.6 months) [64, 65]. More recently, in a randomised trial, addition of vemurafenib to the combination of cetuximab and irinotecan resulted in prolonged PFS [4.4 vs 2.0 months, hazard ratio (HR) 0.42, 95% confidence interval (CI) 0.26–0.66; $p < 0.001$] and a higher disease control rate (67% vs 22%; $p < 0.001$) compared with cetuximab and irinotecan treatment alone in heavily pre-treated patients with *BRAF*-mutant mCRC [66]. The combination of encorafenib (BRAF inhibitor), binimetinib (MEK inhibitor) and cetuximab is being assessed in the BEACON trial and has previously shown encouraging clinical activity in *BRAF* V600E mCRC (ORR: 48%) [67]. Of note, extended (non-V600/non-V600E) *BRAF* mutations may have different clinical implications compared with *BRAF* V600E mutations [68, 69]. It is recommended that *BRAF* mutation status is assessed alongside that of *RAS* for prognostic assessment and/or selection for clinical trials (Table 1) [3, 7]. The predictive potential of *BRAF* mutation status is not yet confirmed.

Regarding the potential of EGFR activation as a biomarker, studies have focused on the expression of EGFR ligands and *EGFR* gene copy number. High expression levels of the EGFR ligands amphiregulin (AREG) and epiregulin (EREG) have positive prognostic value and are associated with a positive response to anti-EGFR therapy [70–72]. For

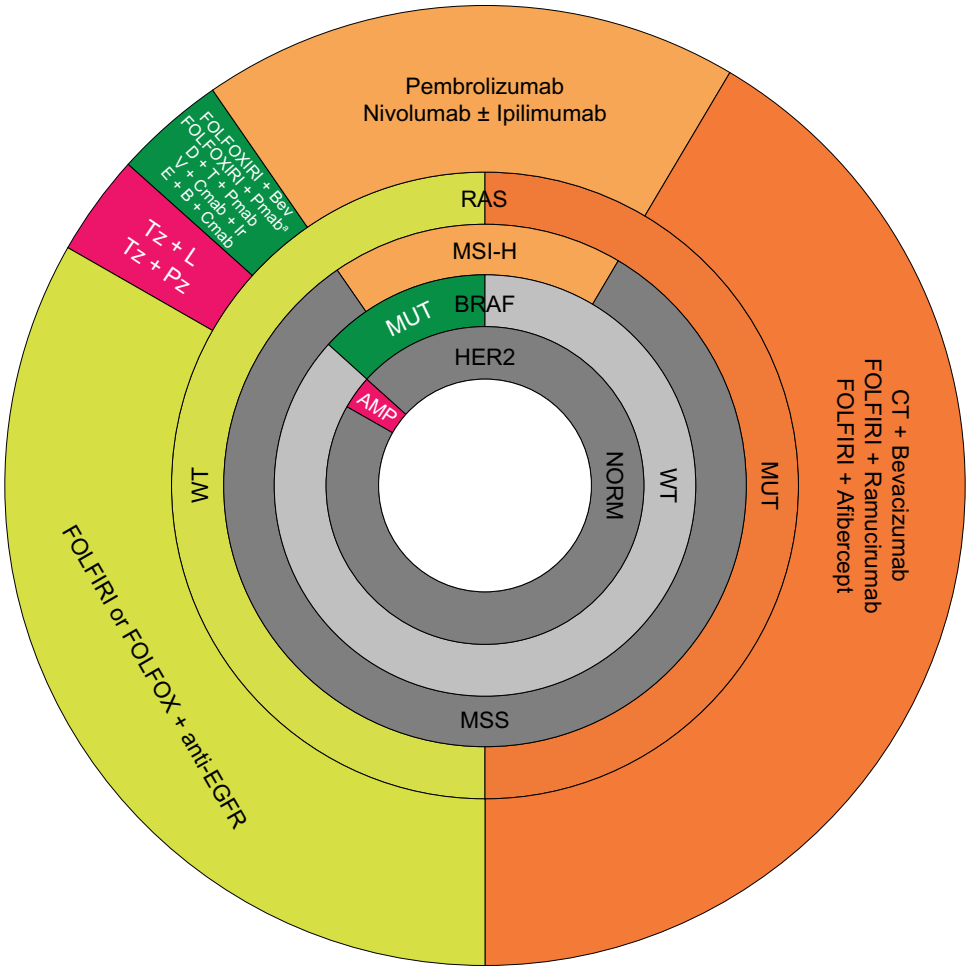

Fig. 2 Possible treatment strategies according to biomarker status in mCRC. [a]Where maximum tumour shrinkage is the goal; further confirmatory data are needed. Colours indicate possible treatment strategies for tumours with amplified HER2 (pink), mutant *BRAF* (green), MSI-H (light orange), WT *RAS*; (yellow) and mutant *RAS* (orange). Grey shading indicates WT/normal expression/MSS with no treatment recommendations. *AMP* amplified, *B* binimetinib, *Bev* bevacizumab, *BRAF* B-rapidly accelerated fibrosarcoma, *Cmab* cetuximab, *CT* chemotherapy, *D* dabrafenib, *E* encorafenib, *EGFR* epidermal growth factor receptor, *FOLFIRI* leucovorin, fluorouracil and irinotecan, *FOLFOX* leucovorin, fluorouracil and oxaliplatin, *FOLFOXIRI* leucovorin, fluorouracil, irinotecan and oxaliplatin, *HER2* human epidermal growth factor 2, *Ir* irinotecan, *L* lapatinib, *mCRC* metastatic colorectal cancer, *MSI-H* microsatellite instability high, *MSS* microsatellite stable, *MUT* mutant, *NORM* normal, *Pmab* panitumumab, *Pz* pertuzumab, *RAS* rat sarcoma, *T* trametinib, *Tz* trastuzumab, *V* vemurafenib, *WT* wild type

example, in *RAS* (*KRAS* and *NRAS*) WT patients receiving anti-EGFR therapy, high AREG and EREG expression correlated with better survival outcomes [73–75]. High *EGFR* gene copy number has also been associated with improved response to anti-EGFR-targeted therapies [76–78], but clinical use of such a biomarker is limited by the rarity of true gene amplification and difficulties in obtaining reproducible results by fluorescence in situ hybridisation assessment [79]. Evaluation of EGFR ligands, *EGFR* gene copy number, or EGFR protein expression is currently not recommended for routine patient management in mCRC (Table 1) [3].

Concerning other components of EGFR downstream signalling pathways, contradictory data are reported for the prognostic and predictive role of *PIK3CA* and *PTEN* mutations in mCRC [80]. As such, according to European and US guidelines there is insufficient evidence for their use as predictive biomarkers for EGFR-antibody therapy (Table 1) [3, 7].

Other promising biomarkers include the receptors HER2 and HER3 (Table 2). Approximately 5% of mCRC tumours are driven by *HER2* amplification or mutation, which can lead to resistance to EGFR-targeted treatment by activating a bypass signalling pathway [81–84]. Although the prognostic role of *HER2* remains uncertain, alterations in this gene have been associated with poorer survival outcomes [81, 85]. There is also a growing interest in HER2 as a

Table 2 Selected trial data for emerging biomarkers of response/resistance to standard treatments in mCRC

Biomarker	References	No. of patients	Prior therapy	Treatment	Key findings
HER2	[83]	135	Anti-EGFR therapy	Anti-EGFR therapy	Median PFS in patients receiving anti-EGFR therapy was significantly shorter in those with amplified compared with non-amplified HER2 tumours (2.9 vs 8.1 months, HR 5.0; $p < 0.0001$). These findings were confirmed in a second cohort: median PFS 2.8 vs 9.3 months (HR 6.6; $p < 0.0001$)
	CA-2008-0012; NCT00853931 [185]	34	CT, Bev	Pmab	The level of HER2 protein expression was significantly associated with resistance to Pmab; HER2 was overexpressed in 4/11 non-responding and 0/21 responding cases ($p = 0.035$)
HER3	PICCOLO; ISRCTN93248876 [91]	308	Fluoropyrimidine ± oxaliplatin ± Bev	Pmab + Ir or Ir alone	High HER3 was predictive of Pmab benefit. In patients with high HER3 expression, median PFS was 8.2 months (Pmab + Ir) vs 4.4 months (Ir) (HR 0.33; 95% CI 0.19–0.58; $p < 0.001$). Patients with low HER3 expression gained no benefit in PFS: 3.3 months (Pmab + Ir) vs 4.3 months (Ir) (HR 0.96; 95% CI 0.67–1.38; $p = 0.84$), with significant interaction ($p = 0.002$). The binary model was also predictive for OS, with significant interaction ($p = 0.01$)
miR-31-3p	[97, 99]	132	FOLFOX/FOLFIRI/ anti-EGFR	NA	miR-31-3p expression level was significantly associated with PFS and OS In one study, statistical models based on miRNA expression discriminated between high and low risk of progression. PFS of high- and low-risk patients was 9 and 35.3 weeks, respectively (HR 4.10, 95% CI 1.3–13.2; $p = 0.018$)
	New EPOC trial; NCT00482222 [98]	149	Adjuvant CT	Cmab + CT or CT alone	Median PFS for mid or high miR-31-3p expression was shorter in the Cmab vs the CT arm (26.7 vs 12.3 months, HR 2.28, 95% CI 1.27–4.09; $p = 0.006$). Low miR-31-3p expressors had similar outcomes irrespective of treatment (HR 1.06, 95% CI 0.43–2.61; $p = 0.9$)
	FIRE-3; NCT00433927 [99]	340	No prior systemic therapy	Cmab + FOLFIRI or Bev + FOLFIRI	Low miR-31-3p expressors had a significantly better OS (HR 0.61, 95% CI 0.41–0.88; $p < 0.01$; median OS: 39.4 vs 27.4 months, respectively), PFS (HR 0.74, 95% CI 0.55–1.00, $p = 0.05$; median PFS: 11.8 vs 10.5 months) and ORR (OR 4.0, 95% CI 1.9–8.2; $p < 0.01$) when treated with FOLFIRI plus Cmab as compared to FOLFIRI + Bev. miR-31-3p is predictive of Cmab effect on OS ($p = 0.07$), PFS ($p = 0.08$) and ORR ($p = 0.06$)
HPP1-methylated free-circulating DNA	AIO-KRK-0207; NCT00973609 [161]	467	NR	Bev + CT or Bev alone or no maintenance	Patients with reduced *HPP1*-methylated free-circulating DNA after administration of combination CT had better OS compared with those with continued detectable levels of *HPP1*-methylated free-circulating DNA ($p < 0.0001$).

Bev bevacizumab, *CI* confidence interval, *Cmab* cetuximab, *CT* chemotherapy, *EGFR* epidermal growth factor receptor, *FOLFIRI* leucovorin, fluorouracil, and irinotecan, *FOLFOX* leucovorin, fluorouracil, and oxaliplatin, *HER* human epidermal growth factor, *HR* hazard ratio, *Ir* irinotecan, *mCRC* metastatic colorectal cancer, *miRNA* microRNA, *NA* not applicable, *NR* not reported, *OR* odds ratio, *ORR* objective response rate, *OS* overall survival, *PFS* progression-free survival, *Pmab* panitumumab

therapeutic target in mCRC (Fig. 2). Dual HER2 blockade with a monoclonal antibody (pertuzumab or trastuzumab) and a tyrosine kinase inhibitor (lapatinib) has been shown to inhibit tumour growth in patient-derived xenografts of HER2-amplified mCRC [86, 87]. Moreover, results from the HERACLES-A Phase II trial showed that dual blockade was efficacious and well tolerated in HER2-positive *KRAS* exon 2 WT mCRC patients refractory to current standard of care [88, 89]. HER2-targeted therapy was also effective in the Phase IIa MyPathway study that involved 57 patients with refractory HER2-amplified/overexpressing CRC treated with trastuzumab plus pertuzumab; the ORR was 32% [90]. Interestingly, this study included patients with *KRAS*-mutated CRC, but efficacy of HER2-directed therapy was notably higher in those with *KRAS*-WT tumour status [90].

With respect to *HER3*, a prospectively planned retrospective biomarker study of pre-treatment samples from the PICCOLO trial showed that patients with *RAS* WT mCRC and high *HER3* mRNA expression benefited markedly from panitumumab treatment, whereas those with low *HER3* mRNA expression did not [91]. There were statistically significant biomarker-treatment interactions for both PFS ($p = 0.001$) and OS ($p = 0.004$) [91].

Some miRNAs have been suggested to predict response to anti-EGFR therapy [92–94]. For instance, high-intensity levels of the Let-7c/miR-99a/miR-125b signature have been associated with longer PFS in *KRAS* WT patients receiving such therapy [95]. Low miR-181a expression has also been associated with poorer outcomes in *KRAS* WT patients undergoing treatment with EGFR-targeting monoclonal antibodies [93, 95], and upregulation of miR-31-5p has been shown to be predictive of shorter PFS in patients with mCRC receiving anti-EGFR treatment [92, 96]. Furthermore, a number of studies have identified miR-31-3p as a promising predictive biomarker for anti-EGFR therapy in *RAS* WT mCRC, with therapeutic benefit potentially restricted to patients with low miR-31-3p expression [97–99].

Finally, primary tumour location, as a surrogate marker for tumour molecular characteristics, is known to affect prognosis and treatment outcomes with anti-EGFR therapy. Left-sided tumours are more prevalent and associated with better prognosis than right-sided tumours [4–6]. Right-sided tumours are more frequently associated with mutations in *BRAF*, *TGFβR2*, and *PI3KCA* and are microsatellite unstable [4, 5, 100]. In contrast, amplification of EGFR and HER2, overexpression of EGFR ligands and chromosomal instability are more common in left- than right-sided tumours [4, 5]. In the first-line setting, anti-EGFR treatment combined with chemotherapy appears to be more effective than bevacizumab in left-sided *RAS* WT mCRC [100]. Moreover, patients with right- versus left-sided tumours benefit less from anti-EGFR therapy [100]. In patients with right-sided tumours, treatment with intensive chemotherapy plus bevacizumab may be more appropriate, although anti-EGFR therapy remains an option to achieve an objective response if cytoreduction is the treatment goal [100]. Furthermore, patients with right-sided tumours appear to benefit more from immunotherapies due to increased antigenic load, although further validation is required [101].

Critically, clinical efficacy of targeted therapy is limited by the development of acquired resistance [102]. A comprehensive analysis of mechanisms of resistance in plasma from a large cohort of patients treated with anti-EGFR therapy showed that the emergence of *RAS* mutations (30%) and *EGFR* extracellular domain (ECD) mutations (25%) were the most frequent mechanisms of resistance [103]. The dynamics of *EGFR* ECD mutations differ from the emergence of *RAS* mutations; patients who experience greater and longer responses to anti-EGFR therapy reportedly develop *EGFR* ECD mutations and patients with shorter PFS seem more likely to develop *RAS* mutations [104]. Mutations in *BRAF*, as well as *MET* and *HER2,* were also detected [103]. Importantly, these biomarkers of resistance appear to be heterogeneous and mostly sub-clonal, which will potentially limit the efficacy of further lines of therapy [103]. Another recent study noted that in patients receiving anti-EGFR therapy and undergoing resection, some patients (19%) gained while others (35%) lost mutations on the resection specimen as compared with previous biopsy, mainly in *RAS*, providing further evidence of intra-tumoural heterogeneity [105]. *RAS* mutations (at biopsy or resection) were associated with worse response and survival compared with tumours that were *RAS* WT [105]. However, in another study, *RAS* mutations that emerged during panitumumab-based treatment (detected by plasma analysis of ctDNA) were not associated with less favourable outcomes [106]. Of note, the emergence of acquired mutations shown to confer resistance can be detected in plasma months before morphological tumour progression [107–109]; the clinical utility of assessing emerging mutations requires further validation.

5 Biomarkers and Anti-angiogenic Therapy in mCRC

The role of angiogenesis in tumourigenesis is shown in Fig. 1. Despite the importance of this process in disease pathology, not all patients with mCRC derive clinical benefit from anti-angiogenic therapy, highlighting the need for biomarkers to ensure treatment is appropriately targeted. However, the discovery of universal predictive biomarkers for anti-angiogenic therapies is challenging, due to host-involvement in angiogenesis.

Many factors have been identified as being associated with better outcomes in patients treated with anti-angiogenic agents, suggesting their potential predictive value, such as

the loss of chromosome 18q11.2–q12.1 [110], the transcription factor homeobox B9 [111, 112], VEGF-D [113, 114], and markers of tumour vasculature immaturity [115]. While VEGF-A was found not to be predictive of anti-angiogenic treatment efficacy in retrospective and prospective series [27, 28], it has been suggested that the VEGF-A splice isoforms 165b and 121 may predict response to bevacizumab [116, 117]. Low levels of hepatocyte growth factor have also been associated with survival benefit from bevacizumab treatment [117]. Further, several miRNAs have been identified as possible biomarkers for anti-angiogenic therapy [118]. For example, high miR-664-3p expression was significantly predictive of improved outcomes in patients with mCRC receiving bevacizumab treatment plus chemotherapy compared with those receiving chemotherapy alone [119]. However, none of these potential biomarkers have acquired sufficient evidence to recommend their use in daily practice and their clinical utility needs to be confirmed in large prospective trials.

6 Microsatellite Instability/Deficient Mismatch Repair Disease

Microsatellite instability (MSI) is a consequence of deficient mismatch repair (dMMR) and serves as a favourable prognostic marker for stage II/III CRC [120–122]. However, the prevalence of dMMR in mCRC is lower (5%) than in the adjuvant setting (around 15%) [123], and data on the prognostic and predictive values of MSI in the metastatic setting are scarce and conflicting [3, 124, 125]. In the metastatic setting, MSI-High (H) tumours are associated with poor prognosis, although *BRAF* mutations are more common in patients with MSI-H tumours versus those with proficient mismatch repair (pMMR) ($p < 0.001$), which may account for this prognosis [126]. While some studies indicate that MSI status does not predict the effect of chemotherapy or targeted therapy in mCRC [127, 128], a recent randomised Phase III trial has found that patients with MSI-H tumours, experienced a longer OS when treated with chemotherapy plus bevacizumab versus cetuximab ($p < 0.001$) [129]. Further prospective studies are warranted. However, MSI status has been shown to be predictive for the use of immunotherapy in the treatment of patients with mCRC (Table 1) [130]. In a Phase II study designed to evaluate the clinical activity of pembrolizumab (programmed cell death ligand 1 inhibitor), the immune-related objective response rate [40% ($n = 4/10$) vs 0% ($n = 0/18$)] and 20-week immune-related PFS rate [78% ($n = 7/9$) vs 11% ($n = 2/18$)] were higher for patients with dMMR versus pMMR CRCs [130]. Based on such early clinical data, nivolumab and pembrolizumab have been approved by the US Food and Drug Administration (FDA) for mCRC patients with MSI-H/dMMR disease that has progressed following treatment with a fluoropyrimidine, oxaliplatin and irinotecan (Fig. 2) [131, 132]. More recently, the FDA approved low-dose ipilimumab in combination with nivolumab for use in these patients based on the CheckMate-142 study, which demonstrated that this combination produces high response rates, encouraging survival outcomes and may provide improved efficacy relative to immuno-monotherapy (Fig. 2) [133, 134]. MSI testing for immune checkpoint inhibitors was included in the most recent ESMO guidelines, prior to FDA approval of these agents [3].

There is an ongoing need to develop new strategies to improve the efficacy of checkpoint inhibitors in microsatellite stable disease. However, the use of combination strategies, such as combining checkpoint inhibitors with MEK inhibitors to increase the number of infiltrating effector lymphocytes, or anti-angiogenic agents for their immunomodulatory properties, have not demonstrated any benefit to date [135–137]. Recent data suggest that inactivation of DNA repair may provide benefit by increasing the tumour neoantigen burden [138], an approach which has the potential to be therapeutically exploited by the use of alkylating agents.

7 Other Emerging Biomarkers for Predicting Therapeutic Response in mCRC

Recent studies have uncovered a number of other potentially important biomarkers for predicting therapeutic response (Table 2). Tumour mutational load, defined as the number of mutations per coding area of a tumour genome, is associated with MSI/MMR status [139]. Some studies have demonstrated that tumour mutational load may be a predictive biomarker for response to chemotherapy and immunotherapy in patients with mCRC; however, the data need to be confirmed in larger studies [129, 140–142]. The relationship between mutations that impair DNA polymerase epsilon (POLE) proofreading and tumour immunogenicity have been explored. In a retrospective analysis of more than 4500 patients with stage II/III CRC, the presence of POLE mutations identified a subset of CRC patients with immunogenic tumours and very good prognosis [143]. The hypermutated phenotype of these tumours suggests that they will be excellent candidates for immunotherapeutic approaches.

Tumours bearing gene fusions, including rearrangements in *RET, ALK, ROS1*, and *NTRK1-2-3,* may represent rare but clinically relevant mCRC subtypes with poor prognosis [144, 145]. Targeted strategies inhibiting RET, ALK, ROS, and TrkA-B-C have demonstrated encouraging results [144–146]; however, mechanisms of resistance may develop and mutations have been observed in the catalytic domain of receptors [147]. Preliminary evidence suggests these fusions may be negative predictive biomarkers for anti-EGFR

therapy [144, 145, 148]. Of note, a recent post hoc analysis of the VALENTINO study evaluating the PRESSING panel, which was created to group rare genomic markers beyond *RAS/BRAF*, including *RET*, *ALK*, *ROS1*, and *NTRK1-2-3*, to predict anti-EGFR resistance [149], found that PRESSING-positive tumours had poorer outcomes compared with PRESSING-negative tumours in patients receiving FOLFOX plus panitumumab followed by maintenance with panitumumab ± 5-FU/leucovorin (PFS 7.7 vs 12.1 months, HR 2.07, 95% CI 1.43–2.99; $p = 0.0001$) [148].

Hypermethylation of CpG islands is frequently observed in CRCs, which are then classified as CpG island methylator phenotype (CIMP) positive [150, 151]. Contradictory data have been reported for the prognostic and predictive role of CIMP status in CRC [152–156]. In patients with stage III CRC treated with oxaliplatin-based adjuvant chemotherapy, CIMP was recently found to be associated with shorter OS (HR 1.46, 95% CI 1.02–1.94; $p = 0.04$) and shorter survival after recurrence (HR 1.76, 95% CI 1.20–2.56; $p < 0.0004$) [157]. Interestingly, there was a non-significant trend for a possible detrimental effect of cetuximab in patients with CIMP-positive tumours [157]. Promoter CpG island hypermethylation of O(6)-methylguanine-DNA-methyltransferase, a DNA repair protein, may predict clinical response to alkylating agents although further research is warranted [158, 159]. A number of other epigenetic prognostic markers have been investigated in CRC [160]. For example, a recent prospective study suggests that *HPP1* methylation may be both a prognostic marker and early marker of response in mCRC [161].

Interest is growing in ctDNA as an analyte for evaluating prognosis and early treatment response [162]. For example, in addition to being of known prognostic value, ctDNA has been proposed as an early marker of response to chemotherapy in patients with mCRC [163, 164]. In a recent prospective study, patients with a high (> 10 ng/mL) versus low (≤ 0.1 ng/mL) ctDNA concentration before initiating first- or second-line chemotherapy had a shorter OS (6.8 vs 33.4 months; adjusted HR 5.64, 95% CI 2.5–12.6; $p < 0.0001$) [164]. Further to this, patients who did not experience 'early normalisation' or an 'early decrease > 80%' of ctDNA concentration after initiation of treatment experienced less benefit from chemotherapy [164].

Finally, classification/stratification systems for CRC, such as consensus molecular subtypes (CMS) and colorectal cancer intrinsic subtypes (CRIS), have been proposed [165, 166]. Both exploit the intrinsic gene signatures specific to CRC cells and may have predictive and prognostic value in mCRC [165–168]. Specifically, CMS has been shown to be prognostic for ORR ($p = 0.023$), PFS ($p < 0.001$), and OS ($p < 0.001$) [167], and a recent small retrospective study suggests that it is also predictive for the efficacy of chemotherapy in mCRC [168]. Further to this, CRIS has been shown to predict response to EGFR-targeting antibodies and to predict disease outcome independently of clinical stage and stromal infiltration [166].

8 Ongoing Molecular-Guided Clinical Trials

Several molecular-guided clinical trials in mCRC are underway (Table 3). FOCUS4, which began recruitment in 2014, is an integrated programme of parallel, molecularly stratified, randomised comparisons for patients with advanced or mCRC [169]. It is derived from a multi-arm, multi-stage design to be adjustable should new biomarker and clinical data arise during the trial, while being cost and time efficient. In this programme, novel agents are tested in patient populations defined by whether their tumours harbour mutations in *BRAF*, *PIK3CA*, *RAS*, and *TP53* or are MSI/dMMR. Its multi-stage design provides an early efficacy signal of the new agents being assessed through a series of pre-planned interim analyses [169]. Other ongoing molecular-guided trials include a Phase III study investigating pembrolizumab versus standard-of-care chemotherapy as first-line therapy for dMMR or MSI-H mCRC (NCT02563002), and Phase II studies investigating atezolizumab and bevacizumab in patients with unresectable mCRC and MSI (NCT02982694), anti-EGFR therapy in mCRC patients with low or high ERCC1 (NCT01703390), treatment with HER2 monoclonal antibodies in HER2-amplified advanced or mCRC (NCT03365882), and Sym004 treatment in chemotherapy-refractory mCRC patients with acquired resistance to anti-EGFR therapy (NCT03549338).

Other clinical trials are exploring re-challenge with anti-EGFR therapy. Dynamic clonal competition leads to a rise in anti-EGFR-resistant mutant clones during anti-EGFR therapy and a decline upon withdrawal of anti-EGFR antibodies [170, 171]. A recent study found that, after discontinuation of anti-EGFR therapy, *RAS* and *EGFR* clones exponentially decayed with an estimated half-life of 3.4 and 6.9 months, respectively [171]. These observations provide a molecular rationale for studies that have proposed re-challenge with cetuximab [172–174] or panitumumab [175, 176], after a previous response to anti-EGFR therapy and may help guide timing of re-challenge therapies. Recent results from the CRICKET trial indicate that re-challenge with cetuximab following acquired resistance is more efficient in patients without *RAS* mutations assessed by ctDNA [177]. These first results suggest that monitoring tumour sensitivity to anti-EGFR agents by iterative ctDNA assessments may soon form part of our daily practice. Additional studies (CHRONOS, NCT03227926; RASINTRO, NCT03259009; FIRE-4, NCT02934529; A-REPEAT, NCT03311750) are investigating different challenge strategies using anti-EGFR

Table 3 Ongoing molecularly guided clinical trials in mCRC

Trial name/identifier	Title	Phase	Status	Molecular selection	Experimental arm	Comparator arm
FOCUS4 EudraCT: 2012-005111-12	Molecular selection of therapy in colorectal cancer: a molecularly stratified randomised controlled trial programme	NR	FOCUS4-A: In development FOCUS4-B: Active, not recruiting FOCUS4-C: Recruiting FOCUS4-D: Active, not recruiting FOCUS4-N: Recruiting	Mutations in *BRAF, PIK3CA, RAS* and *TP53* or MSI/dMMR	Dependent on molecular selection	Dependent on molecular selection
KEYNOTE-177 NCT02563002	A Phase III study of pembrolizumab (MK-3475) vs chemotherapy in microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) stage IV colorectal carcinoma	III	Active, not recruiting	MSI-H/dMMR	Pembrolizumab	Standard of care
NCT02982694	A Phase II open-label study with the anti-PD-L1 atezolizumab monoclonal antibody in combination with bevacizumab in patients with advanced chemotherapy resistant colorectal cancer and MSI-like molecular signature	II	Recruiting	MSI	Atezolizumab and bevacizumab	NA
NCT01703390	Pilot study: biomarker directed treatment in metastatic colorectal cancer	II	Recruiting	ERCC1	ERCC1 low: mFOLFOX6 + cetuximab ERCC1 high: FOLFIRI + cetuximab	NA
S1613 NCT03365882	A randomized Phase II study of trastuzumab and pertuzumab (TP) compared to cetuximab and irinotecan (CETIRI) in advanced/metastatic colorectal cancer (mCRC) with HER-2 amplification	II	Recruiting	*HER2*	Pertuzumab, trastuzumab	Cetuximab, irinotecan hydrochloride
NCT03549338	A Phase II, randomised, open-label, multicentre, three-arm trial of Sym004 versus each of its component monoclonal antibodies, futuximab and modotuximab, in patients with chemotherapy-refractory metastatic colorectal carcinoma and acquired resistance to anti-EGFR monoclonal antibody therapy	II	Active not recruiting	Acquired resistance to anti-EGFR therapy	Sym004	Futuximab or modotuximab
CHRONOS NCT03227926	A Phase II trial of rechallenge with panitumumab driven by RAS clonal-mediated dynamic of resistance	II	Recruiting	*RAS,* progression following anti-EGFR therapy	Panitumumab	NA

Table 3 (continued)

Trial name/identifier	Title	Phase	Status	Molecular selection	Experimental arm	Comparator arm
RASINTRO NCT03259009	Predictive impact of RAS mutations in circulating tumour DNA for efficacy of anti-EGFR reintroduction treatment in patients with metastatic colorectal cancer	NR	Not yet recruiting	*RAS*, progression following anti-EGFR therapy	Anti-EGFR monoclonal antibody	NA
FIRE-4 NCT02934529	A randomised study to assess the efficacy of cetuximab rechallenge in patients with metastatic colorectal cancer (RAS wild-type) responding to first-line treatment with FOLFIRI plus cetuximab	III	Recruiting	*RAS*, progression following FOLFIRI + cetuximab	Cetuximab	Anti-EGFR-free treatment (investigator's choice)
A-REPEAT NCT03311750	Single-arm Phase II study of panitumumab rechallenge in combination with oxaliplatin- or irinotecan-based chemotherapy in patients with RAS wild-type advanced colorectal cancer	II	Recruiting	RAS, progression following anti-EGFR therapy	Panitumumab	NA

BRAF B-rapidly accelerated fibrosarcoma, *dMMR* deficient mismatch repair, *EGFR* epidermal growth factor receptor, *ERCC1* excision repair cross-complementation group 1, *FOLFIRI* leucovorin, fluorouracil, and irinotecan, *FOLFOX* leucovorin, fluorouracil, and oxaliplatin, *HER2* human epidermal growth factor 2, *mCRC* metastatic colorectal cancer, *MSI* microsatellite instability, *MSI-H* microsatellite instability high, *NA* not applicable, *NR* not reported, *PD-L1* programmed cell death ligand 1, *PIK3CA* phosphatidylinositol 3-kinase catalytic subunit alpha, *RAS* rat sarcoma

therapy based on liquid biopsy assessment of dynamic *RAS* clones.

9 Concluding Remarks

Current guidelines regarding biomarkers recommend routinely making treatment decisions based on *RAS*, *BRAF*, or MSI status. In this review, we highlight several other molecular and non-molecular biomarkers that are undergoing clinical testing and we describe their possible clinical relevance (Table 4) as well as highlighting possible treatment strategies according to biomarker status (Fig. 2). For example, *DPYD* genotyping/phenotyping may soon become a standard of care to individualise 5-FU chemotherapy dosing. Furthermore, in addition to assessment of *BRAF* V600E mutations, assessment of *HER2* amplification may be useful to inform physicians of their patients' prognosis and to guide enrolment of patients into ongoing clinical trials dedicated to these rare subgroups. Knowledge of prognostic factors can be used in clinical decision making to determine the goal of treatment and to tailor treatment, for example the selection of adjuvant therapy or level of treatment intensity [3, 178]. Plasma analysis of ctDNA shows promise as a minimally invasive and sensitive method to monitor patient response, including acquired resistance to anti-EGFR agents. However, its utility has to be confirmed and crucially, simple assessment tools and positive controls are required for daily use, before ctDNA testing can become standard practice. Of note, recent studies highlight the use of serial plasma biopsies to assess tumour heterogeneity to further inform treatment decisions [179, 180].

Further clinical validation of many of the biomarkers reviewed here is still required. However, validation and translation of new biomarkers into clinical practice is a complex process involving a number of steps, each with its own set of challenges [181]. Many biomarkers are validated retrospectively, yet such studies can be affected by multiple sources of bias [181]. Thus large-scale prospective trials with well-defined protocols are needed to further identify, develop, and validate biomarkers, to standardise their use in clinical practice and inform treatment options [2, 8]. However, these are costly and time consuming [181]. Employing prospective–retrospective study designs [182] or using biobanks from randomised trials [183] provide alternative options for biomarker discovery [181], and validating molecular-guided clinical trials, including those utilising a multi-arm, multi-stage design for cost and time efficiency, are underway. Of note, the interaction between biomarkers is also likely to be clinically relevant and network biomarkers may provide further prognostic and predictive insight in the future [184].

Table 4 An overview of the potential clinical relevance of the evolving molecular biomarker landscape in mCRC

Biomarker	Clinical relevance[a]	Biomarker type	Clinical implications
DPD (DPYD)	I	Predictive	See Table 1 for current guidance. Testing before fluoropyrimidine administration is not routinely recommended. However, some European countries currently recommend genotype-guided individualised dosing and this may become increasingly universally utilised in the clinic
UGT1A1	I	Predictive	See Table 1 for current guidance
RAS (KRAS, NRAS)	I	Predictive	See Table 1 for current guidance
MSI	I	Predictive and prognostic	See Table 1 for current guidance
BRAF	II	Prognostic; predictive value to be confirmed	See Table 1 for current guidance
CMS	III	Predictive and prognostic	CMS has been shown to be prognostic for response and survival outcomes and predictive for chemotherapy efficacy
CRIS	III	Predictive	CRIS has been shown to predict response to anti-EGFR therapy
HER2	II	Predictive; prognostic value to be confirmed	Alterations in this gene have been associated with poorer survival outcomes. HER2 may become a valuable therapeutic target in mCRC; dual HER2-targeted therapy has demonstrated efficacy
EGFR	III	Predictive	See Table 1 for current guidance
HER3	III	Predictive	High HER3 expression is predictive of anti-EGFR therapy benefit
microRNA	III	Predictive	A number of microRNAs have been identified as promising predictive biomarkers for anti-EGFR therapy
Anti-angiogenic markers	III	Predictive	Many markers have been identified as predictive for response to anti-angiogenic agents; however, their clinical utility needs to be confirmed in large prospective trials
Tumour mutational load	III	Predictive; prognostic value to be confirmed	Tumour mutational load may be a predictive biomarker for response to chemotherapy and immunotherapy
Gene fusions (*RET/ALK/ROS1/NTRK*)	III	Predictive and prognostic	Preliminary evidence suggests that rare gene fusions may be negative predictive biomarkers for anti-EGFR therapy. Targeted strategies inhibiting RET, ALK, ROS and TrkA-B-C have demonstrated encouraging results
CIMP	III	Predictive and prognostic to be confirmed	Data for the prognostic and predictive role of CIMP status in CRC are currently contradictory
HPP1 methylation	IV	Prognostic	Detection of *HPP1* methylation before chemotherapy has been associated with poor survival outcomes
TS	IV	–	See Table 1 for current guidance
ERCC1	IV	–	See Table 1 for current guidance
PIK3CA	IV	–	See Table 1 for current guidance
PTEN	IV	_	See Table 1 for current guidance

BRAF B-rapidly accelerated fibrosarcoma, *CIMP* CpG island methylator phenotype, *CMS* consensus molecular subtypes, *CRC* colorectal cancer, *CRIS* colorectal cancer intrinsic subtypes, *DPD* dihydropyrimidine dehydrogenase, *DPYD* DPD gene, *EGFR* epidermal growth factor receptor, *ERCC1* excision repair cross-complementation group 1, *HER* human epidermal growth factor, *KRAS* Kirsten rat sarcoma viral oncogene, *mCRC* metastatic colorectal cancer, *MSI* microsatellite instability, *NRAS* neuroblastoma RAS, *PIK3CA* phosphatidylinositol 3-kinase catalytic subunit alpha, *PTEN* phosphatase and tensin homolog, *RAS* rat sarcoma, *TS* thymidylate transferase, *UGT1A1* UDP glucuronosyltransferase 1 family, polypeptide A1

[a]I, currently clinically relevant; II, likely to be clinically relevant soon; III may be clinically relevant in the future; IV, not clinically relevant

Overall, we are optimistic that the continued prospective validation of biomarkers along with further developments in patient molecular profiling technologies will help to achieve the goal of true individualised therapy for patients with mCRC.

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