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Review

Treatment sequencing in metastatic colorectal cancer

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Abstract Metastatic colorectal cancer (mCRC) remains incurable in most cases, but survival has improved with advances in cytotoxic chemotherapy and targeted agents. However, the optimal use and sequencing of these agents across multiple lines of treatment is unclear. Here, we review current treatment approaches and optimal treatment sequencing across the first-, second- and third-line settings in mCRC, including biological aspects affecting sequencing and rechallenge. Effective first-line therapy is a key determinant of treatment outcomes and should be selected after considering both clinical factors and biological markers, notably *RAS* and *BRAF*. The second-line regimen choice depends on the systemic therapies given in first-line. Anti-angiogenic agents (e.g. bevacizumab, ramucirumab and aflibercept) are indicated for most patients, whereas epidermal growth factor receptor (EGFR) inhibitors do not improve survival in the second-line setting. Molecular profiling is important in third-line treatment, with options in *RAS* wild-type patients including EGFR inhibitors (cetuximab or panitumumab), regorafenib and trifluridine/tipiracil. Immunotherapy with pembrolizumab or nivolumab ± ipilimumab may be considered for patients with high microsatellite instability disease. Targeting *HER2/neu* amplification shows promise for the subset of CRC tumours displaying this abnormality. Sequencing decisions are complicated by the potential for any treatment break or de-escalation to evoke a distinct clinical progression type. Ongoing trials are investigating the optimal sequencing and timing of therapies for mCRC. Molecular profiling has established new targets, and increasing knowledge of tumour evolution under drug pressure will possibly impact on sequencing.
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1. Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed tumours worldwide and a leading cause of cancer death [1]. Recent advances in therapy and multidisciplinary care have led to significant improvements in survival, but a cure is not possible for most patients with metastatic CRC (mCRC). A major challenge has been the finding that anti-epidermal growth factor receptor (EGFR) antibodies are rendered ineffective by alterations in *RAS* genes (*KRAS*/*NRAS* exons 2–4) [2–4]. This is particularly important given that ~50% of colorectal tumours are known to have a mutated *RAS* gene [5], and this patient population has benefitted significantly less from improvements in treatment. Current options for mCRC include cytotoxic chemotherapy and targeted therapies across multiple lines of treatment [6]. However, the optimal use and sequencing of these agents has yet to be determined [7]. Despite favourable outcomes reported from first-line trials [4,8], the effect on survival of continuing therapy beyond the specified study treatment remains uncertain and can only be estimated from trials of second-line and subsequent therapy.

In this review, we discuss current treatment approaches in the first-, second- and third-line settings in mCRC, together with recent developments in treatment sequencing across multiple lines of treatment, and their future implications.

2. Treatment lines and treatment efficacy

First-line therapy is the key determinant of successful systemic treatment in mCRC, because it has the longest treatment duration, is the most effective in terms of response and progression-free survival and is the only line of therapy that all treated patients are sure to receive (Table 1) [9–20]. For the majority of patients, effective first-line therapy is therefore crucial, whereas subsequent therapy addresses only a subset of patients, who may present with a reduced tolerance of toxic side-effects, resulting in short treatment durations compared to first-line therapy [21]. This is particularly important in patients who may become candidates for metastatic resection and receive initial combination therapy [22]. However, if no potential curative path is reached by first-line therapy, the question of drug sequencing gains in importance.

3. First-line treatment

Multiple factors influence the choice of first-line treatment in mCRC, including both clinical factors and molecular markers.

3.1. Clinical factors

The precise extent of disease (differentiating oligo- vs. poly-metastatic disease as well as the distinct organs involved) is an important factor in determining the choice of first-line treatment [22]. Metastatic resection, alone or combined with other interventions, should be aimed for if complete removal or ablation of all tumour is possible [22,23]. Conversion to resectable disease may be achieved using combination regimens with favourable response rates [22–24].

The primary tumour location affects both the prognosis of patients with mCRC and the activity of targeted therapies, in particular EGFR antibodies. Patients with left-sided tumours gain a clear benefit from initial EGFR-based therapy, whereas these agents are less likely to benefit those with right-sided tumours apart from initial response rate [25,26]. This interaction, observed in patients with *RAS* wild-type tumours, should be taken into account when deciding on first-line therapy.

In patients who are unfit or unwilling to receive combination chemotherapy, especially the elderly, treatment options may include a dose-adjusted regimen of fluoropyrimidine plus oxaliplatin [27] or alternatively a combination of fluoropyrimidine plus bevacizumab [28–31].

3.2. Molecular markers

The presence of certain molecular markers helps guide treatment in mCRC. As noted, patients with *RAS*-mutant CRC are unlikely to benefit from EGFR-targeted therapy, and accumulating evidence suggests that patients with a *BRAF*-mutant tumour do not gain a substantial benefit from EGFR antibodies, although data are limited by sample size and differing treatment background [22,32]. Accordingly, both the European

Table 1
Treatment efficacy across the continuum of care in colorectal cancer[a].

Outcome parameter	First-line [16,45,49,74,114–117]	Second-line [11,15,17–19,39–41]	Third-line [9,12,14,20,118]
Response rates	38–65%	5–36%	1–31%
Disease control rate	81–90%	59–86%	41–75%
Progression-free survival	9–12 months	4–7 months	2–5 months

[a] Ranges for efficacy data are taken from the targeted/experimental treatment arms of all studies that reported the specified end-point (for epidermal growth factor receptor trials, results are given for *RAS* wild-type subsets where applicable). Disease control rates were calculated as response (complete or partial) plus stable disease, which was assessed at varying time points.

Society for Medical Oncology (ESMO) and the United States (US) National Comprehensive Cancer Network (NCCN) guidelines recommend testing for *RAS* (*KRAS* exon 2 and non-exon 2 *NRAS*) and *BRAF* prior to first-line therapy [22,23]. For patients with wild-type *RAS*, the treatment goal is prolongation of life and/or secondary resection using a cytotoxic doublet with an anti-EGFR therapy, a doublet with bevacizumab or a triplet with or without bevacizumab [22,23]. Of course, these guidelines undergo re-evaluation, and the NCCN guidelines currently include primary tumour location as an additional factor in decision-making for first-line treatment [23]. A chemotherapy doublet with bevacizumab is also an option for patients with *RAS*-mutant tumours [22]. For those with *BRAF*-mutant tumours, data are less robust, and any regimen might be acceptable, while in fit patients, a triplet chemotherapy with fluorouracil, folinic acid, oxaliplatin and irinotecan (FOLFOXIRI) plus bevacizumab may be a reasonable choice [22,33]. In patients with good performance status and an urgent need for cytoreduction, FOLFOXIRI combined with an EGFR antibody may also represent a highly active option [34,35]. Results from key clinical studies of targeted therapies in the first-line setting are shown in Table 2.

4. Second-line treatment

Typical second-line chemotherapy options for mCRC patients include fluorouracil, folinic acid and irinotecan (FOLFIRI) and fluorouracil, folinic acid and oxaliplatin (FOLFOX) [22,23], depending on the systemic therapies given in the first-line setting [36]. The choice of first-line therapy initiates the treatment sequence, leading to algorithms based on varying levels of clinical evidence, as shown in Box 1.

Whereas the chemotherapy sequence of FOLFOX then FOLFIRI or vice versa does not seem to significantly impact outcome [37], it is unclear to what extent this can be extrapolated to the antibody era. Various antiangiogenic agents and EGFR antibodies may be combined with chemotherapy doublets (Table 3), but evidence remains limited for some of these sequential treatments. Of note, four phase 3 studies of anti-angiogenic agents have shown prolongation of overall survival in patients (some of them previously treated with vascular endothelial growth factor [VEGF]-targeted agents) in the second-line setting [17–19,38]. In contrast, all studies investigating EGFR antibodies in the second-line setting have to date failed to demonstrate any such survival benefit [39–41]. In the second-

Table 2
Key trials of targeted therapies in the first-line setting; only phase 3 studies with contemporary infusional combination regimens supporting ESMO treatment guidelines are shown.

Trial	Treatment arms	Median survival, months (hazard ratio; 95% CI)		Reference
		PFS	OS	
EGFR antibodies[a]				
CRYSTAL	Cetuximab + FOLFIRI versus FOLFIRI	9.9 versus 8.7 (HR 0.68, 0.50–0.94; $P = 0.02$)	24.9 versus 21.0 (HR 0.84, 0.64–1.11; NS)	Van Cutsem 2009 [48]
PRIME	Panitumumab + FOLFOX-4 versus FOLFOX-4	9.6 versus 8.0 (HR 0.80, 0.66–0.97; $P = 0.02$)	23.9 versus 19.7 (HR 0.83, 0.67–1.02; $P = 0.072$)	Douillard 2010 [49]
FIRE-3	Cetuximab + FOLFIRI versus bevacizumab + FOLFIRI	10.0 versus 10.3 (HR 1.06, 0.88–1.26; $P = 0.55$)	28.7 versus 25.0 (HR 0.77, 0.62–0.96; $P = 0{\cdot}017$)	Heinemann 2014 [45]
CALGB 80405	Cetuximab + chemo versus bevacizumab + chemo (physician's choice of FOLFIRI or mFOLFOX-6)	10.5 versus 10.6 (HR 0.95, 0.84–1.08; $P = 0.45$)	30.0 versus 29.0 (HR 0.88, 0.77–1.01; $P = 0.08$)	Venook 2017 [117]
VEGF antibodies				
Saltz	Bevacizumab + FOLFOX-4 or XELOX versus placebo + FOLFOX-4 or XELOX	9.4 versus 8.0 (HR 0.83, 0.72–0.95; $P = 0.0023$)	21.3 versus 19.9 (HR 0.89, 0.76–1.03; $P = 0.0769$)	Saltz 2008 [16]
TRIBE	Bevacizumab + FOLFOXIRI versus bevacizumab + FOLFIRI	12.1 versus 9.7 (HR 0.75, 0.62–0.90; $P = 0.003$)	29.8 versus 25.8 (HR 0.80, 0.65–0.98; $P = 0{\cdot}03$)	Cremolini 2015; Loupakis 2014 [8,74]
ITACa	Bevacizumab + chemo versus chemo (physician's choice of FOLFIRI or FOLFOX-4)	9.6 versus 8.4 (HR 0.86, 0.70–1.07; $P = 0.182$)	20.8 versus 21.3 (HR 1.13, 0.89–1.43, $P = 0.317$)	Passardi 2015 [119]

CI, confidence interval; EGFR, epidermal growth factor receptor; FOLFIRI, fluorouracil, folinic acid and irinotecan; FOLFOX, fluorouracil, folinic acid and oxaliplatin; FOLFOXIRI, fluorouracil, folinic acid, oxaliplatin and irinotecan; HR, hazard ratio; OS, overall survival; NS, not significant; PFS, progression-free survival; VEGF, vascular endothelial growth factor; XELOX, capecitabine plus oxaliplatin; ESMO, European Society for Medical Oncology.

[a] Results are presented for patients with *RAS* wild-type tumours only.

Box 1. Summary of European Society for Medical Oncology treatment recommendations in the second-line setting

- Patients progressing on first-line oxaliplatin-containing regimens, with or without bevacizumab, should be considered for treatment with irinotecan-based therapy consisting of FOLFIRI or single-agent irinotecan plus the appropriate antibodies (see below) [22].
- Patients progressing on first-line irinotecan containing regimens should be considered for treatment with FOLFOX/XELOX, alone or in combination with antibodies [22].
- Patients who received first-line EGFR-based therapy should be switched to a VEGF-targeted agent [22].
- Patients who started with VEGF-based therapy are recommended to continue with this strategy, with or without a change of agent within this class. Alternatively, although the scientific rationale is limited, if the tumour is *RAS* and *BRAF* wild type (and left primary tumour), an EGFR-based regimen can be considered [22,39–41,46,47].
- If first-line therapy was based on the FOLFOXIRI regimen, second-line options could include reintroduction after maintenance of the initial therapy or a less intensive regimen (EGFR antibody plus irinotecan, regorafenib or trifluridine/tipiracil) [22].

line setting, VEGF-targeted agents, which include bevacizumab, ramucirumab and aflibercept, are thus indicated for most patients [22,23]. The choice of three approved agents and the absence of head-to-head trials comparing bevacizumab with the newer alternatives make determining the optimal antiangiogenic agent in the second-line setting particularly challenging [42]. Bevacizumab has been evaluated in combination with both FOLFOX and FOLFIRI [11,17], whereas ramucirumab and aflibercept have only been evaluated in combination with FOLFIRI and should be used accordingly [18,19,22,23]. Therefore, patients who progress rapidly on first-line bevacizumab- and oxaliplatin-containing regimens can be considered for treatment with aflibercept or ramucirumab but only in combination with FOLFIRI. Levels of VEGF-D may also be useful in determining whether to continue with bevacizumab or switch to an alternative agent; in particular, high VEGF-D levels after progression on bevacizumab appeared to correlate with ramucirumab efficacy in the phase 3 RAISE trial [43].

Limited data are also available to support a treatment sequence of EGFR antibody, followed by VEGF-targeted agents. In the absence of evidence from prospectively designed trials, data from first-line studies suggest that EGFR-based first-line therapy might create a favourable precondition for second-line therapy, including VEGF-targeted antibodies [44]. In particular, data from FIRE-3 support the sequence of an EGFR antibody followed by a VEGF-targeted agent based on the observed success of second-line therapy following cetuximab in this study (Fig. 1) [4,44,45].

Overall, the available evidence suggests that VEGF-targeted agents should generally be preferred over EGFR antibodies in second-line therapy. This assumption is also supported by two randomised phase 2 trials comparing bevacizumab continuation versus switching to EGFR antibodies in second-line, with no reported benefit for switching antibodies [46,47]. Also, regimens containing oxaliplatin plus an EGFR antibody have never been prospectively tested in pretreated patients, further increasing the difficulty of evaluating different sequences starting with irinotecan-based regimens.

Prospective trials investigating treatment across lines of therapy are currently underway, including STRATEGIC-1 (ClinicalTrials.gov Identifier: NCT01910610), FIRE-4 (NCT02934529) and TRIBE-2 (NCT02339116).

5. Role of molecular subgroups in second-line therapy

As discussed above, second-line therapy may be considered the least attractive setting for EGFR-targeted agents based on the available evidence from first-line and later-line trials. Consequently, it is questionable whether the corresponding biomarker (i.e. *RAS* status) has an impact on the choice of second-line therapies [9,14,39–41,45,48–51].

While it is generally assumed that VEGF-targeted agents, which significantly improve overall survival in second-line trials, can be used irrespective of *KRAS*/*RAS* mutational status, taken as a whole published evidence could suggest that patients with *KRAS*/*RAS*-mutant mCRC benefit less from second-line anti-angiogenesis than those with non-mutated tumours. Although none of the individual trials identified a significant interaction, retrospective subgroup testing cannot exclude such an interaction. Table 4 provides an overview of molecular subgroups in second-line trials using VEGF-targeted agents.

Some evidence suggests that patients with refractory, *BRAF*-mutant mCRC might benefit from BRAF-targeted regimens, which typically consist of a BRAF inhibitor, an EGFR antibody, and sometimes a third agent that may be a targeted molecule (e.g. mitogen-activated protein kinase kinase (MEK) inhibitors) or a chemotherapeutic agent (i.e. irinotecan) [52–56].

6. Third-line treatment and beyond

For patients receiving third-line CRC treatment, molecular profiling of the cancer and consideration of clinical trial enrolment are important aspects of management [23]. According to ESMO guidelines, cetuximab or panitumumab should be considered in *RAS* wild-type and *BRAF* wild-type patients not previously treated with

Table 3
Review of recommended targeted therapies in the second-line setting according to ESMO and NCCN guidelines [22,23].

Trial	Treatment Arms	Median survival, months (hazard ratio, 95% CI)		Reference
		PFS	OS	
VEGF antibodies				
ECOG E3200	Bevacizumab + FOLFOX-4 versus FOLFOX-4 versus bevacizumab alone (no prior bevacizumab)	7.3 versus 4.7 versus 2.7 (HR for bevacizumab + FOLFOX-4 versus FOLFOX-4: 0.61; $P < 0.0001$)	12.9 versus 10.8 versus 10.2 (HR for bevacizumab + FOLFOX-4 versus FOLFOX-4 0.75; $P = 0.0011$)	Giantonio 2007 [11]
TML (ML18147, AIO KRK 0504)	Bevacizumab + CT versus CT in patients progressing after 1L bevacizumab (CT: standard 2L regimens based on fluoropyrimidines plus oxaliplatin or irinotecan)	5.7 versus 4.1 (HR 0.68, 0.59–0.78; $P < 0.0001$)	11.2 versus 9.8 (HR 0.81, 0.69–0.94; $P = 0.0062$)	Bennouna 2013 [17]
BEBYP	Bevacizumab + mFOLFOX-6/FOLFIRI versus mFOLFOX-6/FOLFIRI in patients progressing after 1L bevacizumab	6.8 versus 5.0 (HR 0.70, 0.52—0.95; P = 0.010)	14.1 versus 15.5 (HR 0.77, 0.56—1.06; P = 0.043)	Masi 2015 [38]
EAGLE	Bevacizumab (5 versus 10 mg/kg) + FOLFIRI in patients progressing after 1L bevacizumab	6.1 versus 6.4 months (HR 0.95, 0.75–1.21; P = 0.676)	16.3 versus 17.0 (HR 1.08; 0.75–1.57; P = 0.667)	Iwamoto 2015 [120]
VELOUR	Aflibercept + FOLFIRI versus FOLFIRI (prior bevacizumab: 30% of patients)	6.90 versus 4.67 (HR 0.76, 0.66–0.87; $P < 0.0001$)	13.50 versus 12.06 (HR 0.82, 0.71–0.94; $P = 0.0032$)	Van Cutsem 2012 [18]
RAISE	Ramucirumab + FOLFIRI versus placebo + FOLFIRI in patients progressing after 1L bevacizumab	5.7 versus 4.5 (HR 0.79, 0.70–0.90; $P < 0.0005$)	13.3 versus 11.7 (HR 0.84, 0.73–0.98; $P = 0{\cdot}0219$)	Tabernero 2015 [19]
EGFR antibodies				
EPIC	Cetuximab + irinotecan versus irinotecan alone (no prior anti-EGFR therapy)	4.0 versus 2.6 (HR 0.69, 0.62–0.78; $P \leq 0.0001$)	10.7 versus 10.0 (HR 0.98, 0.85–1.11; $P = 0.71$)	Sobrero 2008 [39]
PICCOLO[a]	Panitumumab + irinotecan versus irinotecan (no prior anti-EGFR therapy)	Median PFS not reported (HR 0.78, 0.64–0.95; $P = 0{\cdot}015$)	10.4 versus 10.9 (HR 1.01, 0.83–1.23; $P = 0{\cdot}91$)	Seymour 2013 [40]
Study 181[a]	Panitumumab + FOLFIRI versus FOLFIRI (no prior anti-EGFR therapy)	6.7 versus 4.9 (HR 0.82, 0.69–0.97; $P = 0.023$)	14.5 versus 12.5 (HR 0.92, 0.78–1.10; $P = 0.37$)	Peeters 2014 [41]

1L, first-line; 2L, second-line; CI, confidence interval; CT, chemotherapy; EGFR, epidermal growth factor receptor; FOLFIRI, infusional fluorouracil, folinic acid and irinotecan; FOLFOX, infusional fluorouracil, folinic acid and oxaliplatin; HR, hazard ratio; OS, overall survival; PFS, progression-free survival; VEGF, vascular endothelial growth factor; ESMO, European Society for Medical Oncology; NCCN, National Comprehensive Cancer Network.
[a] Patients with *KRAS* wild-type tumours.

EGFR antibodies [22]. Either regorafenib or the antimetabolite trifluridine/tipiracil (TAS-102) is recommended in patients previously treated with fluoropyrimidines, oxaliplatin, irinotecan and bevacizumab, as well as in *RAS* wild-type patients previously treated with EGFR antibodies [22]. Similarly, NCCN guidelines recommend cetuximab or panitumumab, preferably in combination with irinotecan, in *KRAS*/*NRAS* wild-type patients not previously treated with EGFR antibodies, with regorafenib and trifluridine/tipiracil as alternatives [23].

Unfortunately, the survival benefit obtained with third-line CRC treatment in phase 3 trials is modest. In the CORRECT trial of regorafenib monotherapy, the overall survival benefit for regorafenib versus placebo was only 1.4 months (median 6.4 versus 5.0 months; hazard ratio [HR] 0.77; 1-sided $P = 0.0052$) [12]. Patients assigned to trifluridine/tipiracil in the RECOURSE trial had a survival benefit of 1.8 months compared to placebo (median 7.1 versus 5.3 months; HR 0.68; 1-sided $P < 0.001$) and limited symptomatic toxicity [20]. Regarding the choice of regorafenib versus trifluridine/tipiracil, the drugs can be used in either order and based on the preference patients and physicians [22,23].

Therefore, differences in the mechanism of action and, more importantly, the safety profile of available third-line/further-line mCRC therapies may guide treatment selection for individual patients when quality of life gains importance as a treatment aim. For instance, EGFR inhibitors are associated with toxicities including rash and diarrhoea in tissues expressing EGFR [57], and multikinase inhibitors can cause hand-foot skin reactions, rash, fatigue, diarrhoea and hypertension [58] and antimetabolites are associated with bone marrow suppression and gastrointestinal toxicities [59].

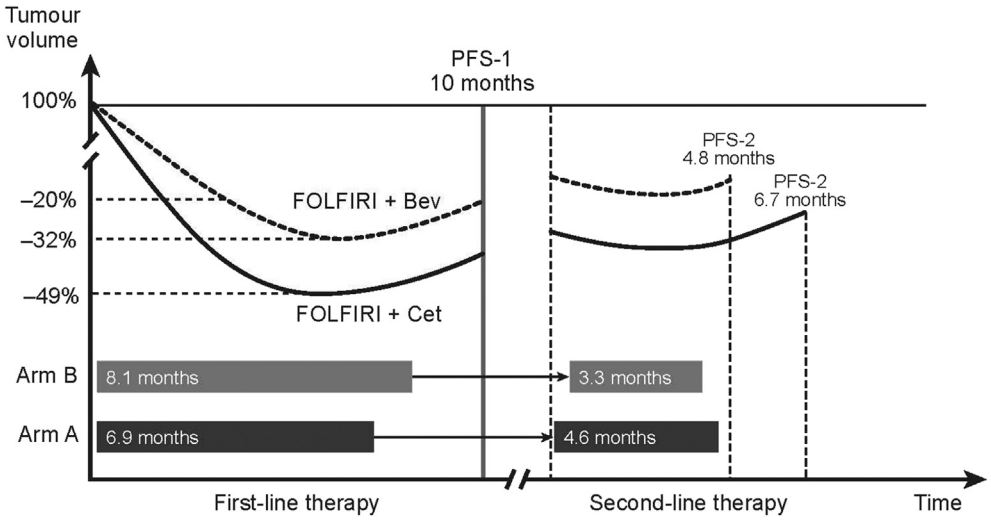

Fig. 1. Schematic model of potential association between treatment duration, depth of response, progression-free survival and subsequent therapy in patients treated with FOLFIRI plus either cetuximab (Arm A) or bevacizumab (Arm B) in FIRE-3 [4,44,45]. Bev, bevacizumab; Cet, cetuximab; FOLFIRI, infusional fluorouracil, folinic acid and irinotecan, PFS, progression-free survival from start of first-line [PFS-1] or second-line [PFS-2] therapy. Arm A: FOLFIRI/cet, Arm B: FOLFIRI/bev. Observations from FIRE-3 suggest that despite comparable PFS, different first-line therapies can invoke heterogeneous preconditions for second-line therapy based on depth of response, treatment-duration (and resulting toxicity) and additional biologic factors (not shown). Horizontal bars represent treatment duration and curves represent tumour burden. Data are reported for the extended *RAS* wild-type population and values represent medians.

Immunotherapy is increasingly used to treat tumours with deficient DNA mismatch repair (MMR), which occurs in patients with Lynch syndrome or as a result of somatic tumour mutations. Compared with MMR-proficient tumours, those with MMR deficiency show high microsatellite instability (MSI-high) and carry many more mutations and were thus hypothesised to be a target for immunotherapy. Phase 2 trials of the programmed cell death protein-1 inhibitors pembrolizumab and nivolumab and the combination of nivolumab plus ipilimumab have demonstrated promising response rates and also favourable early survival signals in pretreated patients [60–62]. Although confirmation in phase 3 studies is awaited, the NCCN guidelines recommend pembrolizumab or nivolumab as treatment options in second-line and beyond for patients with deficient MMR/MSI-high mCRC [23].

The explosion in molecular profiling of tumours has resulted in identification of new targets and combination therapies. Among these, *HER2/neu* amplification has emerged as a promising therapeutic target for the 1.6–6.3% fraction of CRC displaying this molecular abnormality [63]. Patients with *KRAS* wild-type, *HER2/neu*-overexpressing mCRC have been treated with the anti-HER2 antibody trastuzumab and the dual EGFR/HER2 kinase inhibitor lapatinib as part of the HERACLES trial, in which HER2 status was determined using the CRC-specific HERACLES Diagnostic Criteria [64].

Table 4
Survival benefit by molecular subgroup in second-line trials of VEGF-targeted agents.

Study	Arms	Subgroup	Sample size	Hazard ratio for death (95% CI)	Reference
RAISE	FOLFIRI + ramucirumab	*KRAS* WT	267	0.82 (0.67–0.999)	Tabernero 2015 [19]
	FOLFIRI		275		
	FOLFIRI + ramucirumab	*KRAS*m	269	0.89 (0.73–1.09)	
	FOLFIRI		261		
VELOUR	FOLFIRI + aflibercept	*RAS* WT	218	0.70 (0.50–0.97)	Wirapati 2017 [121]
	FOLFIRI				
	FOLFIRI + aflibercept	*RAS*m	264	0.93 (0.70–1.23)	
	FOLFIRI				
TML	CT + bevacizumab	*KRAS* WT	151	0.69 (0.53–0.90)	Kubicka 2013 [122]
	CT		165		
	CT + bevacizumab	*KRAS*m	164	0.92 (0.71–1.18)	
	CT		136		

CI, confidence interval; CT, chemotherapy; FOLFIRI, infusional fluorouracil, folinic acid and irinotecan; FOLFOX, infusional fluorouracil, folinic acid and oxaliplatin *(K)RAS*m, mutant *KRAS* or extended *RAS*; WT, wild-type.

Thirty-three patients had been enrolled and were evaluable for response. Complete responses were observed in two patients (6%) and partial responses in eight patients (24%), giving an overall response rate of 30% [65,66]. The efficacy of a HER2-directed therapy has been confirmed in the MyPathway phase 2a multiple basket study, where the CRC cohort was expanded to enrol 34 patients with HER2-amplified/overexpressed mCRC who had exhausted standard treatments [67,68]. Patients received a combination of trastuzumab and pertuzumab, achieving an overall response rate of 38%, stable disease for <4 months in 11% of patients. Ongoing studies are evaluating the potential of combining the HER2/HER3 dimerisation inhibitor pertuzumab with trastuzumab [66] or trastuzumab emtansine (DM1) [69] in this patient population. Finally, emerging actionable molecular alterations in mCRC include rare gene fusions of neurotrophic tyrosine kinase receptor, type 1 (*NTRK1*) and anaplastic lymphoma kinase (*ALK*) that can be tackled by specific inhibitors [70,71] and ring-finger protein 43 (*RNF43*) mutations or R-spondin 3 (*RSPO3*) gene fusions leading to dysregulation of Wnt signalling susceptible to porcupine inhibitors [72].

7. Clinical understanding of sequencing and timing of therapies

The optimal sequencing of therapy centres on first-line decisions, as all other lines and combinations depend on the choice of up-front treatment. In patients with untreated mCRC, therapeutic goals may range from cure to palliation. The choice of first-line therapy therefore takes into account both the treatment goal and the molecular subtype of the tumour [22]. In this context, the sequence of systemic treatment is of particular importance both in patients with secondary resection of metastases (with a high likelihood of relapse) as well as in patients who, for whatever reason, miss the opportunity of secondary resection and receive multiple lines of therapy. Whereas perspectives and treatment goals are heterogeneous in first-line therapy, second and subsequent lines of therapy generally aim to prolong life at a reasonable cost of toxicity.

The current understanding of sequential treatment in mCRC is limited by the fact that treatment until progression without breaks or de-escalation appears impossible in a substantial fraction of patients (>50% of patients in some studies) receiving first-line therapy [16,44]. Some recent studies have therefore predefined or explored maintenance regimens to enable treatment until progression and spare toxicity [73,74]. In particular, de-escalation/re-escalation strategies have been evaluated for oxaliplatin-based regimens to address the cumulative toxicity of this drug, with results indicating that this strategy does not impair clinical outcomes [75,76]. In recent years, randomised studies have also investigated bevacizumab-based maintenance therapy following oxaliplatin-based regimens, a strategy that has succeeded in delaying disease progression, although a clear effect on overall survival has not been demonstrated [73,77]. EGFR-based maintenance strategies following oxaliplatin-based combination therapy have likewise been explored in randomised phase II trials without providing any firm conclusions regarding optimal maintenance [32,78,79]. Overall, significant controversies remain concerning the oncologic value (i.e. no clear survival benefit) versus toxicity and quality-of-life effects of maintenance therapy, especially as compared with treatment breaks. However, any de-escalation or treatment break evokes a distinct clinical progression type (i.e. progression during full treatment,

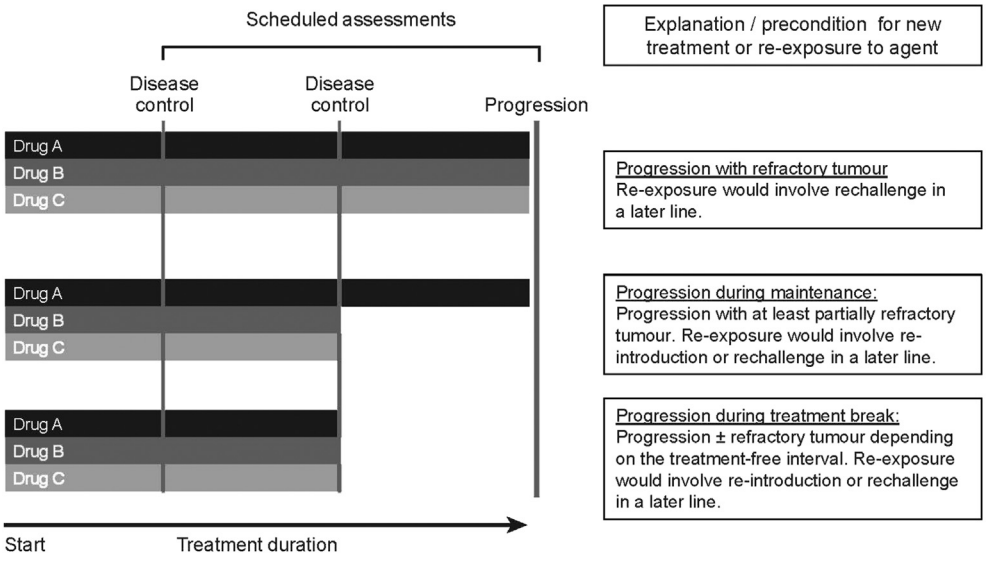

Fig. 2. Clinical understanding of the types of progression encountered in first-line therapy.

de-escalated treatment or treatment holidays) (Fig. 2). This purely clinical information may impact the choice and options for later lines of therapy. In particular, re-introduction of previously de-escalated agents can easily be justified when progression was not observed in the context of the respective drug, but only within the same treatment line. Besides the clinical understanding of progression, emerging data illuminate how systemic treatment of CRC results in altered tumour biology and consecutively acquired molecular resistance to a given therapy (see also Section 8.2) [80–83]. Finally, studies evaluating a biomarker-driven maintenance treatment for first-line mCRC are ongoing (ClinicalTrials.gov Identifier: NCT02291289) and will potentially reshape the concept of therapeutic sequence for selected molecular subsets of patients, such as those with tumours harbouring *ERBB2* amplification, *BRAF* mutation or MSI-high status.

It could be assumed that any treatment and any result of therapy (e.g. resistance while on treatment, progression during a treatment break or de-escalated therapy or even tumour response with unacceptable toxicity) creates a distinct precondition for subsequent therapy that can be understood on a clinical level and might also be reflected by measurable or as yet unmeasurable changes in molecular tumour features. Both the heterogeneity of clinical progression and our limited understanding of the residual sensitivity (to regimens or their individual components) of tumours following progression highlight the need for molecular characterisation to guide decisions in the sequential treatment of mCRC.

8. Biological aspects concerning sequencing and rechallenge

8.1. Transition from first- to second-line treatment

Preclinical data have provided insight on how VEGF- and EGFR-targeted therapeutics can modulate the cancer environment and thus impact the optimal sequencing of these agents, while clinical trials have established that concomitant administration of VEGF and EGFR inhibitors increases toxicity with no improvement in survival [84]. For the transition from first- to second-line treatment, it is well established that bevacizumab exposure in CRC is associated with an increase in serum levels of VEGF-A [85,86] as well as several cytokines and angiogenic factors (including fibroblast growth factor, hepatocyte growth factor, placental growth factor, stromal-derived factor-1 and macrophage chemoattractant protein-3) [87]. Recently, a retrospective analysis of baseline plasma samples from the phase 3 VELOUR trial of FOLFIRI ± aflibercept as second-line therapy showed that prior first-line bevacizumab was associated with elevated VEGF-A levels (mean: 753–763 pg/mL for bevacizumab pretreated patients compared with 149–165 pg/mL in patients with no prior bevacizumab) [88]. The biological impact of this finding was assessed in *RAS* wild-type CRC cells, demonstrating that VEGF-A induced resistance to cetuximab by upregulation of VEGFR-2/Stat-3 signalling and phosphorylation of ERK1/2 [86]. Together, these preclinical data suggest that the activity of EGFR-targeted agents may be reduced when administered after exposure to bevacizumab.

On the other hand, exposure to EGFR-targeted monoclonal antibodies in first-line is associated with the emergence/expansion of *RAS* mutations [81]. As noted, KRAS activation induces VEGF expression, and CRC cell lines with KRAS activation and constitutive upregulation of the *VEGF* gene can be inhibited by specific inhibitors of RAS downstream signals, such as PI3K and AKT inhibitors [89]. This latter point might underlie the observation of reduced activity of VEGF-targeted therapeutics following exposure to anti-EGFR agents. However, CRC cancer cell lines and xenografts developing resistance to EGFR-targeted agents have constitutively high expression of VEGF, VEGFR-1 and placenta growth factor [90]. This suggests a role for VEGF-targeted agents that have inhibitory activity toward other members of the VEGF family, such as aflibercept [91], or blockade of the VEGF pathway through multikinase agents that include a VEGFR-targeted component (such as regorafenib) [90,92] as rescue therapy after development of resistance to EGFR inhibitors.

8.2. Chemo-refractory setting

Few data are available to guide the transition from second to later lines of treatment in CRC, and the dynamic evolution of tumours in this heavily pretreated setting is difficult to assess, as unstable tumour genomes alter substantially over the course of treatment because of clonal selection of resistant cells during chemotherapy and targeted treatments [93]. However, molecular diagnostic techniques, such as liquid biopsy for analysis of circulating tumour DNA (ctDNA), have evolved [94], and translational research in this field may provide some indirect guidance for positioning of available drugs in the sequence of CRC therapy. In particular, studies using liquid biopsy for longitudinal monitoring have revealed clonal evolution during anti-EGFR therapy, showing that mutant *RAS* clones arise in blood during EGFR blockade [95] and decline upon withdrawal of treatment [81].Anti-EGFR pressure can give rise to multiple emergent circulating mutations of the MAPK pathway within the same patient [94,99], in what has been referred to as a ‘war of clones’. However, clinical monitoring of these emergent *RAS* mutations in circulating tumour DNA is premature, as a direct impact on response to EGFR-targeted agents has not been

established [96], and studies are needed to determine clinically relevant and validated thresholds for emergent ctDNA *RAS* mutations. Further, mutations in the EGFR extracellular domain are uniquely induced by drug pressure and, therefore, only observed in the setting of secondary resistance [97,98]. Tabernero et al. performed a retrospective analysis of *KRAS*, *PIK3CA* and *BRAF* mutations in DNA obtained from the plasma of 503 patients enrolled in the CORRECT trial of regorafenib. The authors identified a subgroup of patients whose archival tumour tissue was *KRAS* wild-type, but whose plasma DNA was *KRAS* mutant, confirming the acquisition of *KRAS* mutations as a mechanism of resistance to EGFR-antibody therapy, since all patients with apparent acquired *KRAS* mutations had received EGFR antibody treatment before enrolment. In this setting, the benefit of regorafenib was confirmed irrespective of historical or plasma *KRAS* status.

This increase in knowledge of tumour evolution under drug pressure has potential clinical implications for the sequencing of agents in later lines of CRC treatment. First, available data suggest that treatment with kinase inhibitors, including a VEGFR-targeted component such as regorafenib, in the advanced-line setting is not jeopardised by the acquisition of secondary *KRAS* mutations induced by EGFR inhibitors [93,99]. Second, the plasticity of CRC cells and the dynamic clonal competition that takes place during EGFR-targeted therapy and on withdrawal of EGFR blockade might be clinically exploited, as the decline of mutated *KRAS* clones can be associated with a renewed response to EGFR antibodies [81]. This observation provides a molecular rationale for studies that proposed rechallenge with cetuximab [100,101] or panitumumab [102,103] after a previous response to anti-EGFR therapy. Additional studies are investigating different rechallenge strategies using anti-EGFR therapies (Table 5). Third, genotyping secondary mutations of the EGFR extracellular domain at resistance through solid or liquid biopsy might also have a therapeutic impact. As an example, the induction of the S492R mutation in the extracellular domain of the EGFR with cetuximab therapy but not with panitumumab [104] supports the rationale for anti-EGFR rechallenge with panitumumab in cetuximab pretreated patients who bear this mutation, since it is not thought to confer cross-resistance [97,98]. New-generation EGFR inhibitors, such as the anti-EGFR antibody mixture Sym004, can overcome cetuximab/panitumumab resistance mediated by some of these EGFR mutations [105], even though most CRC patients with EGFR extracellular domain mutations harbour concomitant RAS-RAF-MEK alterations that are predicted to drive resistance to an EGFR antibody mixture alone [106].

9. Future perspectives

For the transition from first-line to second-line treatment, most preclinical evidence concerning the interaction between VEGF and EGFR pathways upon pharmacological inhibition indicates a reduced activity of EGFR-targeting antibodies following exposure to bevacizumab [107], whereas the reverse strategy may lead to favourable outcomes [44]. On the other hand, for patients starting with chemotherapy in combination with bevacizumab, using VEGF-targeted agents beyond and after progression is an established strategy [108], but further research is needed to determine the optimal timing of the switch from bevacizumab to drugs with broader activity such as aflibercept or regorafenib. Although preclinical studies demonstrate that continued antiangiogenic activity will require inhibition of alternative angiogenic pathways or of additional tyrosine kinases, specific biomarkers to guide the decision of whether to switch to broader VEGF inhibitors or continue with bevacizumab treatment are still lacking. Concerning the transition from second to later lines of treatment, expanding preclinical knowledge through the use of liquid biopsy for ctDNA monitoring has opened several scenarios for translation into the clinic. Future studies will elucidate how the plasticity of CRC cells and the dynamic clonal competition that takes place during EGFR-targeted therapy may be exploited, either by using rechallenge strategies or by switching to alternative EGFR-targeted drugs or to agents targeting other specific subclones upon resistance.

Table 5
Ongoing clinical trials assessing a rechallenge strategy with EGFR-targeted agents in mCRC.

Trial name and ID	Phase	Treatment	Selection criteria
NCT03087071 (Cohort 3)	2	Panitumumab	*KRAS*, *NRAS* and *BRAF* wild-type status; no *EGFR* S492R or other ectodomain mutations in circulating free tumour DNA Prior therapy: fluoropyrimidine, irinotecan and oxaliplatin; cetuximab with evidence of clinical benefit (CR, PR or prolonged SD for ≥16 weeks)
FIRE-4 (NCT02934529, 2014-003787-21[a])	3	Cetuximab	*RAS* wild-type status; first-line therapy: FOLFIRI plus cetuximab, with SD ≥ 6 months or better
CHRONOS (NCT03227926, 2016-0902597-12[a])	2	Panitumumab	*RAS* and *BRAF* wild-type status; first-line therapy: PR or better on irinotecan-based chemotherapy (FOLFIRI or FOLFOXIRI) plus cetuximab

[a] EudraCT number. CR, complete response; EGFR, epidermal growth factor receptor; mCRC, metastatic colorectal cancer; PD, progressive disease; PR, partial response; SD, stable disease.

Finally, the advent of genomic analysis has generated new possibilities for evaluating off-label targeted therapies in refractory cancers and for expediting enrolment in studies with matched targeted therapeutics [109,110]. However, the clinical utility of this approach is controversial [60,111]. These advances also pose great challenges to clinicians and pathologists by requiring familiarity with genomic techniques and data as well as the integration of cancer genomics scientists and bioinformaticians in the tumour board. Ongoing studies will determine the true potential of tumour genomics to benefit clinical care, including IMPACT 2 (ClinicalTrials.gov Identifier: NCT02152254), the US National Cancer Institute's MATCH trial (NCT02465060) [112] and specifically for CRC, the ATTACC screening protocol (NCT01196130). Randomised studies investigating immuno-oncologic therapies for MSI-high/deficient MMR mCRC are expected to define the best line and treatment sequence in this subgroup of patients.

10. Conclusions

Upfront testing of molecular markers (*RAS*/*BRAF*) plus consideration of primary tumour locations enables personalised medicine in the first-line therapy of mCRC. In contrast, molecular markers currently do not impact significantly on the choice of second-line therapy, for which the available evidence suggests that VEGF-targeted agents provide a significant survival benefit and are suitable options, irrespective of molecular subtype and pre-treatment. Third-line therapies in mCRC are associated with modest survival gains and potential morbidity; the risks versus benefits of these therapies should be discussed in detail with the patient before initiation of treatment. Consideration should be given to molecular profiling of the MMR status of the tumour, given the compelling evidence that these tumours are vulnerable to immunotherapy with checkpoint inhibitors and also to clinical trial enrolment, especially for *BRAF*- and *HER2*-directed combinations. Rarely, gene fusions can be found in mCRC and should not be neglected because of their potential actionability.

Conflict of interest statement

D.P.M. has had advisory roles with and/or honoraria from Amgen, Merck, Roche, Bristol-Myers Squibb, Sirtex, Merck Sharp & Dohme, Servier, Pfizer, Boehringer-Ingelheim, Taiho, travel support from Amgen, Roche, Merck, Bristol-Myers Squibb, Servier, Taiho and research grant from Amgen, Merck, Roche. S.P. has nothing to disclose. A.S.-B. is a member of advisory boards for Amgen, Bayer and Sanofi. GI connect is supported by an Independent Educational Grant from Bayer.

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