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REVIEW ARTICLE

Optimizing biologic sequencing in metastatic colorectal cancer: first line and beyond

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ABSTRACT

Significant advances in the treatment of metastatic colorectal cancer (mCRC) since the early 2000s have led to improved clinical outcomes, including overall survival (OS). When fluorouracil was the sole treatment agent for mCRC, OS in phase III studies was approximately 12 months. Now, in 2019, the median OS (mOS) in the most recent mCRC clinical trials has been approaching 3 years. The biologic agents that target the vascular endothelial growth factor (VEGF), epithelial growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), PD-1, CTLA-4, NTRK, and BRAF pathways play important roles in the mCRC treatment algorithm, given their significant—sometimes dramatic—activity. Emerging data indicate that the choice of a specific biologic at a particular time (line of treatment) for specific patient populations (based on tumour characteristics) is critical. In the present review, we discuss the available evidence for optimal biologic sequencing in the management of mCRC.

Key Words Colorectal cancer, biologics, treatments

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INTRODUCTION

Worldwide, colorectal cancer (CRC) is the 2nd most commonly diagnosed cancer in men and the 3rd most commonly diagnosed in women. In 2018, 1.8 million new cases, with more than 860,000 deaths, were estimated[1]. In the United States, CRC is the 3rd most frequently diagnosed cancer overall, with 145,600 new cases of CRC expected to be diagnosed in 2019[2].

More than 20% of patients with CRC present with synchronous metastatic disease at their initial diagnosis, and 50%–60% of patients develop metachronous metastases[3–6]. Approximately 56% of patients with CRC will ultimately die from their cancer[7]. Major advances in the treatment of metastatic CRC (mCRC) since the early 2000s significantly improved overall survival (OS) in those patients. Mortality from CRC has been declining tremendously (almost 35% from 1990 to 2007 and 53% from 1970 to 2016) in the United States[2,8]. Those declines are largely attributable to earlier diagnosis because of screening tests and to improved treatment options, including new systemic chemotherapy agents (capecitabine, oxaliplatin, irinotecan, trifluridine/tipiracil) and biologic agents targeting vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), PD-1, CTLA-4, MEK, BRAF, and NTRK. How the treatment of mCRC is optimized, especially by choice and sequencing of biologics, is critical to maximizing the benefits. In the present review, we discuss the role of biologics in the treatment of mCRC.

REVIEW

First-Line Treatment

VEGF Inhibitors

Vascular endothelial growth factor was first identified and isolated in the late 1980s and is recognized as an essential regulator of normal and abnormal blood vessel growth[9,10]. Later, the VEGF family expanded to include the prototype member VEGF (VEGF-A), placental growth factor, VEGF-B, VEGF-C, and VEGF-D[11–14]. Among those factors, VEGF-A constitutes the rate-limiting step in controlling blood vessel growth that includes tumour angiogenesis.

Bevacizumab is a humanized anti-VEGF monoclonal antibody that binds to and neutralizes all VEGF-A isoforms and its proteolytic fragments[15,16]. The AVF2107g trial was the first phase III clinical study to demonstrate the survival benefit of adding bevacizumab to chemotherapy[17] (Table I). Compared with IFL and placebo, adding bevacizumab to

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TABLE I Key phase III studies for biologics in metastatic colorectal cancer

Reference (study name)	Patients *(n)*	Treatment arms	Median survival (months)		Hazard ratio
			Progression-free	Overall	
Hurwitz *et al.*, 2004[17]	402	IFL plus bevacizumab	10.6	20.3	0.66
(AVF2107g)	411	IFL	6.2	15.6	$p<0.001$
Saltz *et al.*, 2008[18]	700	FOLFOX4 or XELOX, both plus bevacizumab	9.4	21.3	0.89
(NO16966)	701	FOLFOX4 or XELOX	8.0	19.9	$p=0.077$
Sobrero *et al.*, 2008[19]	648	Irinotecan plus cetuximab	4.0	10.7	0.975
(EPIC)	650	Irinotecan	2.6	10.0	$p=0.71$
Maughan *et al.*, 2011[20]	362	FOLFOX or XELOX, both plus cetuximab	8.6	17.0	1.04
(COIN)	367	FOLFOX or XELOX	8.6	17.9	$p=0.67$
Van Cutsem *et al.*, 2011[21]	316	FOLFIRI plus cetuximab	9.9	23.5	0.796
(CRYSTAL)	350	FOLFIRI	8.4	20.0	$p=0.0093$
Tveit *et al.*, 2012[22]	97	FLOX	8.7	22.0	1.14
(NORDIC VII)	97	FLOX plus cetuximab	7.9	20.1	$p=0.48$
	109	Intermittent FLOX plus cetuximab	7.5	21.4	
Van Cutsem *et al.*, 2012[23]	612	FOLFIRI plus aflibercept	6.90	13.5	0.817
(VELOUR)	614	FOLFIRI	4.67	12.06	$p=0.0032$
Bennouna *et al.*, 2013[24]	409	Chemotherapy plus bevacizumab	5.7	11.2	0.81
(ML18147)	411	Chemotherapy	4.1	9.8	$p=0.0062$
Cunningham *et al.*, 2013[25]	140	Capecitabine plus bevacizumab	9.1	20.7	0.79
(AVEX)	140	Capecitabine	5.1	16.8	$p=0.78$
Douillard *et al.*, 2013[26]	325	FOLFOX4 plus panitumumab	9.6	23.9	0.83
(PRIME)	331	FOLFOX4	8.0	19.7	$p=0.072$
Grothey *et al.*, 2013[27]	500	Regorafenib	1.9	6.4	0.77
(CORRECT)	253	Placebo	1.7	5.0	$p=0.0052$
Heinemann *et al.*, 2014[28]	297	FOLFIRI plus cetuximab	10.0	28.7	0.77
(FIRE-3)	295	FOLFIRI plus bevacizumab	10.3	25.0	$p=0.017$
Loupakis *et al.*, 2014[29]	252	FOLFOXIRI plus bevacizumab	12.1	31.0	0.79
(TRIBE)	256	FOLFIRI plus bevacizumab	9.7	25.8	$p=0.054$
Peeters *et al.*, 2014[30]	303	FOLFIRI plus panitumumab	6.7	14.5	0.92
(20050181)	294	FOLFIRI	4.9	12.5	$p=0.37$
Tabernero *et al.*, 2015[31]	536	FOLFIRI plus ramucirumab	5.7	13.3	0.844
(RAISE)	536	FOLFIRI	4.5	11.7	$p=0.0219$
Venook *et al.*, 2017[32]	578	FOLFOX or FOLFIRI, both plus cetuximab	10.5	30.0	0.88
(CALGB 80405)	559	FOLFOX or FOLFIRI, both plus bevacizumab	10.6	29.0	$p=0.08$
Qin *et al.*, 2018[33]	193	FOLFOX4 plus cetuximab	9.2	20.7	0.76
(TAILOR)	200	FOLFOX4	7.4	17.8	$p=0.02$
Cremolini *et al.*, 2019	340	FOLFOXIRI plus bevacizumab reintroduction	19.1[a]	27.6	0.81
(TRIBE2)[34]	339	FOLFOX plus bevacizumab, then FOLFIRI plus bevacizumab	16.4[a]	22.6	$p=0.033$
Kopetz *et al.*, 2019[35]	224	Encorafenib plus binimetinib plus cetuximab	NR	9.0	0.52[b]
(BEACON)	221	Irinotecan or FOLFIRI, both plus cetuximab	NR	5.4	$p<0.0001$
	220	Encorafenib plus cetuximab	NR	8.4	0.60[c]
					$p=0.0003$
Sastre *et al.*, 2019[36]	172	FOLFOXIRI plus bevacizumab	12.4	21.7	$p=0.862$
(VISNU-1)	177	FOLFOX plus bevacizumab	9.3	17.6	

[a] For PFS2, defined as the time from randomization to death or to disease progression on any treatment given after 1st disease progression.
[b] Encorafenib–binimetinib–cetuximab arm compared with the irinotecan–cetuximab arm.
[c] Encorafenib–cetuximab arm compared with the irinotecan–cetuximab arm.
IFL = irinotecan, fluorouracil, leucovorin; FOLFOX = bolus and infusional fluorouracil, leucovorin, oxaliplatin; XELOX = capecitabine, oxaliplatin; FOLFIRI = fluorouracil, leucovorin, irinotecan; FOLFOXIRI = fluorouracil, leucovorin, oxaliplatin, irinotecan; FLOX = bolus fluorouracil, leucovorin, oxaliplatin; NR = not reported.

the IFL regimen (irinotecan–fluorouracil–leucovorin) significantly improved median OS (mOS), response rate (RR), and median duration of response. Subsequently, in 2004, the U.S. Food and Drug administration (FDA) approved bevacizumab to be used in combination with intravenous fluorouracil-based chemotherapy as a first-line treatment for patients with mCRC.

Meanwhile, the phase III N9741 trial revealed the clinical benefit in mOS, median progression-free survival (mPFS), and RR of FOLFOX treatment (infusional fluorouracil–leucovorin–oxaliplatin) compared with IFL[37]. Those results led to the widespread adoption of FOLFOX instead of IFL as the backbone chemotherapy regimen to be combined with bevacizumab.

However, in the NO16966 study comparing FOLFOX [or CAPOX (capecitabine–oxaliplatin)] with and without bevacizumab in the first-line treatment of unresectable mCRC, bevacizumab was associated with only a modest 1.4-month mPFS benefit (study primary endpoint) and a modest mOS benefit of 1.4 months, which was not statistically significant[18]. One explanation for those results was the relatively short exposure of the patients to bevacizumab in the study (only 29% of patients in the bevacizumab arm received treatment until disease progression).

No randomized trials comparing FOLFIRI (fluorouracil–leucovorin–irinotecan) with FOLFIRI plus bevacizumab have been reported, but a meta-analysis involving 3502 patients reported that, with FOLFIRI–bevacizumab treatment, the RR was 51.4%; the mPFS, 10.8 months; and the mOS, 23.7 months[38].

The open-label randomized phase III AVEX study compared the efficacy and safety of capecitabine–bevacizumab with capecitabine only in elderly patients (>70 years) with untreated mCRC, enrolling 140 patients into each arm[25]. In the capecitabine–bevacizumab arm, mPFS was significantly longer. The study demonstrated the efficacy and safety of bevacizumab in addition to single-agent fluoropyrimidine chemotherapy in the first-line setting and showed that it is a good option for patients who are not candidates for a doublet chemotherapy backbone regimen.

The phase III TRIBE trial randomized 508 treatment-naïve patients with mCRC to FOLFIRI or FOLFOXIRI (fluorouracil–leucovorin–oxaliplatin–irinotecan), both plus bevacizumab[29]. In the FOLFOXIRI–bevacizumab arm, mPFS, RR, and mOS were observed to be improved. Although the data were encouraging, the advantage of upfront triplet chemotherapy compared with a sequential strategy of doublets (FOLFOX, FOLFIRI) was uncertain, especially given the toxicities associated with intensified treatment. Thus, the phase III TRIBE2 study was conducted to confirm the benefits of a first-line triplet regimen with bevacizumab. The 679 patients with unresectable mCRC who were enrolled into TRIBE2 were randomized to either FOLFOX–bevacizumab followed by FOLFIRI–bevacizumab after disease progression (arm A) or to FOLFOXIRI–bevacizumab followed by a reintroduction of the same regimen after disease progression (arm B)[34]. First-line treatments were administered for up to 8 cycles, followed by fluorouracil–bevacizumab until disease progression. The primary endpoint was PFS2, defined as either death or the time from randomization to disease progression on any treatment given after the 1st disease progression. At a median follow-up of 30.6 months, arm B showed a significant advantage in PFS2 (19.1 months vs. 16.4 months) and mOS (27.6 months vs. 22.6 months). Similarly, the phase III VISNU-1 trial compared FOLFOXIRI–bevacizumab with FOLFOX–bevacizumab in the first-line setting for patients with mCRC who had 3 or more baseline circulating tumour cells[36]. The FOLFOXIRI–bevacizumab arm was associated with a statistically significant improvement in mPFS, the primary endpoint (12.4 months vs. 9.3 months), and a numerically higher mOS (21.7 months vs. 17.6 months, $p = 0.862$).

Several meta-analyses have confirmed the mPFS and mOS benefit of bevacizumab in the first-line treatment of mCRC, although subgroup analysis has suggested that the bevacizumab-related survival benefit is observed only when bevacizumab is combined with irinotecan-based chemotherapy[39–42]. It is widely accepted that the addition of bevacizumab to first-line chemotherapy offers a modest clinical benefit. The side effects associated with bevacizumab include hypertension, thromboembolic events, bleeding, proteinuria, wound complications, and gastrointestinal perforation, but this agent is well tolerated in general.

EGFR Inhibitors

Epidermal growth factor receptor plays an important role in CRC initiation and progression, and EGFR overexpression is detected in 49%–82% of CRCs[43,44]. Cetuximab and panitumumab are monoclonal antibodies against the extracellular domain of the receptor and inhibit its downstream signalling pathways. The RAS/RAF/MAPK pathway is downstream of EGFR, and its mutations are predictive for a lack of benefit from EGFR inhibitors[26,45,46]. It has become clear that patients with mCRC having *RAS (KRAS, NRAS)* mutations will not benefit from treatment with an EGFR inhibitor[47]. Evidence has also strongly suggested that *BRAF* V600E–mutated mCRC (even if *RAS* wild-type) is unlikely to respond to an EGFR inhibitor[48,49]. It is crucial to perform an extended *RAS* mutation test *(KRAS* or *NRAS* exons 2–4) and a *BRAF* mutation test before starting EGFR inhibitor treatment in mCRC.

The phase III CRYSTAL trial investigated the efficacy of cetuximab–FOLFIRI as a first-line treatment for mCRC[50]. Patients were randomized to FOLFIRI with or without cetuximab. In the initial report, the addition of cetuximab to FOLFIRI was associated with only a modest improvement in PFS, without a benefit in OS. However, in the updated analysis, the addition of cetuximab to FOLFIRI in patients with *KRAS* wild-type mCRC was associated with significant improvements in mOS, mPFS, and RR[21].

Cetuximab was also tested in combination with FOLFOX in first-line treatment for mCRC. In the randomized phase II OPUS trial, adding cetuximab to FOLFOX was associated with an increased RR and mPFS, but without a mOS benefit, in patients with *KRAS* wild-type disease[51,52]. Likewise, adding cetuximab to FOLFOX or XELOX (capecitabine–oxaliplatin) in the phase III COIN study did not demonstrate a benefit in mOS or mPFS for the patients with *KRAS* wild-type disease[20]. An improvement in mPFS was seen in the FOLFOX subgroup, but not in the XELOX subgroup. The phase III NORDIC VII trial, in which cetuximab was added to the FLOX

(fluorouracil–oxaliplatin) regimen, also did not show a PFS or OS benefit for cetuximab[22].

However, the most recently reported phase III open-label randomized TAILOR study clearly demonstrated the benefit of adding cetuximab to FOLFOX in the first-line setting[33]. In that study, 393 patients with mCRC *(KRAS* or *NRAS* exons 2–4 wild-type) were treated with FOLFOX with or without cetuximab. Adding cetuximab significantly improved the mPFS (primary study endpoint, 9.2 months vs. 7.4 months) and mOS (20.7 months vs. 17.8 months). The efficacy of FOLFOX–cetuximab was also confirmed in the phase III Cancer and Leukemia Group B/SWOG 80405 trial[32]. The difference between those studies raised the possibility that the chemotherapy backbone, especially the fluoropyrimidine formula, might be critical, and capecitabine-based chemotherapy was not listed for combination with cetuximab in the U.S. National Comprehensive Cancer Network guideline. Another interesting finding was that less oxaliplatin-associated peripheral neuropathy was seen in the cetuximab combination group. That observation suggested that cetuximab might have neuroprotective effects[20,22,51,52].

Panitumumab was tested in combination with FOLFOX in the phase III PRIME study[53]. In patients with *KRAS* wild-type mCRC, the addition of panitumumab to FOLFOX treatment was associated with a significant improvement in mPFS (9.6 months vs. 8.0 months), but the mOS improvement did not reach statistical significance (23.9 months vs. 19.7 months). Interestingly, panitumumab seemed to be detrimental when used in *KRAS*-mutant mCRC; however, the mechanism is still unclear. Nonetheless, that observation confirmed the importance of a comprehensive *RAS* mutation panel test before treatment with an EGFR inhibitor.

Cetuximab and panitumumab can both cause infusion-related reactions (more common in cetuximab), acneiform dermatitis, diarrhea, stomatitis, and hypomagnesemia. Skin toxicities are the most common side effects with EGFR inhibitors. Pre-emptive treatment, including the use of skin moisturizers, sunscreen, topical steroids, and doxycycline, can significantly mitigate the skin toxicities[54]. In general, cetuximab and panitumumab are considered interchangeable in the first-line treatment for mCRC.

Choosing Between VEGF and EGFR Inhibitors in the First-Line Setting

The phase II PEAK trial compared FOLFOX–panitumumab with FOLFOX–bevacizumab in the first-line treatment of patients with *KRAS* exon 2 wild-type mCRC[55]. The mPFS and mOS were found to be longer in the panitumumab arm in the subset of 170 patients who were found to have wild-type *KRAS* and *NRAS*.

The multicentre open-label randomized phase III German FIRE-3 trial compared the efficacy of FOLFIRI–cetuximab with that of FOLFIRI–bevacizumab[28], enrolling 593 patients with *KRAS* exon 2 wild-type mCRC. The study returned a negative result because the objective response rate (ORR), the primary endpoint of the study, was 62% in the cetuximab group compared with 58% in the bevacizumab group ($p = 0.18$). The mPFS was also similar in the cetuximab and bevacizumab groups (10.0 months vs. 10.3 months). However, the mOS was significantly better in the cetuximab arm than in the bevacizumab arm (28.7 months vs. 25.0 months). In a subsequent analysis of the 400 patients with *KRAS* and *NRAS* wild-type tumours, the ORR was significantly higher in the cetuximab group (72% vs. 56%). The mOS was also significantly longer in the cetuximab group (33 months vs. 25 months)[56]. Further retrospective analysis revealed that the benefit of cetuximab over bevacizumab was limited to patients with a left-sided primary tumour (mOS: 38.3 months vs. 28 months); in right-sided primary tumours, bevacizumab was better (mOS: 23 months vs. 18.3 months)[57].

A similar study (Cancer and Leukemia Group B/SWOG 80405) conducted in the United States enrolled 1137 patients with *KRAS* exon 2 wild-type mCRC and treated them with FOLFOX or FOLFIRI, plus either cetuximab or bevacizumab[32]. The mOS (study primary endpoint) and mPFS in the cetuximab arm were similar to those in the bevacizumab arm (mOS: 30 months vs. 29 months; mPFS: 10.5 months vs. 10.6 months). In the all *RAS* wild-type patients, mOS was better in cetuximab-treated left-sided primary tumours (37.5 months vs. 32.1 months), but bevacizumab seemed to be more beneficial in right-sided primary tumours (24.5 months vs. 16.4 months)[58].

A meta-analysis of the three foregoing studies revealed that, in *RAS* wild-type left-sided colon cancer, the survival benefit was significantly greater with an EGFR inhibitor than with bevacizumab (hazard ratio: 0.71; $p = 0.0003$) and that patients with right-sided primary colon cancer benefited from bevacizumab, with a trend toward longer survival (hazard ratio: 1.3; $p = 0.081$)[59].

Based on those data, it would be reasonable to consider an EGFR inhibitor for patients with *KRAS, NRAS,* and *BRAF* wild-type left-sided mCRC, while starting bevacizumab for right-sided tumours. However, patient preference, quality of life, and side effect profiles have to be considered too. Bevacizumab is generally easier to tolerate, without the skin rashes typically associated with EGFR inhibitors and with a mOS similar to that in the Cancer and Leukemia Group B 80405 study. It is still reasonable to consider bevacizumab in the first-line setting for left-sided disease, using an EGFR inhibitor in later lines.

Subsequent-Line Treatment

VEGF Inhibitors

The ML18147 study and the BEBYP trial both demonstrated a mOS benefit by continuing bevacizumab beyond disease progression[24,60]. The randomized open-label phase III E3200 trial showed a mOS benefit by adding bevacizumab to FOLFOX treatment after disease progression on an irinotecan-based first-line treatment[61]. Based on the data from those trials, continuing or starting bevacizumab after disease progression is known to be beneficial.

Other VEGF inhibitors include ziv-aflibercept, which is a recombinant protein designed to trap VEGF and inhibit angiogenesis. The VELOUR study demonstrated its activity in second-line treatment for mCRC[23]. Ramucirumab, a human monoclonal antibody targeting VEGF receptor 2, demonstrated an OS benefit in the RAISE study in the second-line setting[31]. However, these newer VEGF-targeted agents have undergone no head-to-head comparisons or produced dramatic survival benefits. Bevacizumab

is still the most commonly used VEGF inhibitor in the second-line setting.

Regorafenib, which also targets the VEGF receptor, is an oral multikinase inhibitor with antiangiogenesis effects, but low specificity. The phase III international CORRECT study demonstrated a 1.4-month OS benefit for regorafenib compared with best supportive care in heavily pretreated patients with mCRC[27]. The common side effects with regorafenib include hand–foot syndrome, fatigue, diarrhea, and hypertension. The initial approved dose of regorafenib was 160 mg daily, 21 days on and 7 days off, but most patients were found to have difficulty tolerating treatment. The phase II regorafenib dose optimization study, REDOS, showed that, in terms of the percentage of patients who initiated a third cycle of regorafenib (43% vs. 24%), weekly regorafenib dose escalation from 80 mg to 160 mg daily for the first cycle of treatment is superior to starting with a dose of 160 mg daily[62,63]. In addition, a trend toward improved survival was observed in the dose escalation arm compared with the standard dosing arm (9.0 months vs. 5.9 months).

EGFR Inhibitors

In contrast to the situation with bevacizumab, no evidence supports continuation of an EGFR inhibitor after disease progression. However, there is strong evidence for introducing one either as a single agent or combined with chemotherapy after disease progression.

The 20050181 study confirmed a PFS benefit for panitumumab in the second-line setting, although the mOS benefit was not statistically significant[30]. In an open-label phase III study, panitumumab also demonstrated single-agent activity in mCRC after disease progression on oxaliplatin- and irinotecan-based chemotherapy[64]. The retrospective subset analysis from that study after *KRAS* exon 2 mutation testing revealed that the panitumumab benefit was seen only in patients who were *KRAS* exon 2 wild-type[65].

In the EPIC study, no OS benefit was associated with cetuximab in the second-line setting, but mPFS and RR were significantly improved[19]. That finding might reflect the fact that patients were enrolled to the study without their *KRAS* status being known. As a single agent (compared with best supportive care), cetuximab was associated with significantly prolonged mOS and PFS. Subset analysis later revealed that patients who were *KRAS* exon 2 wild-type benefited from cetuximab (mOS: 9.5 months vs. 4.8 months; mPFS: 3.7 months vs. 1.9 months); patients with a *KRAS* mutation did not (hazard ratio: 0.97)[66].

Consequently, adding an EGFR inhibitor to treatment in patients with *KRAS, NRAS,* and *BRAF* wild-type mCRC is recommended for consideration if the patient has not been exposed to the agent before.

Biologics in Special Populations

Deficient Mismatch Repair or High Microsatellite Instability mCRC: Deficiencies in the cellular mismatch repair system (dMMR) lead to DNA replication errors and high microsatellite instability (MSI-H), resulting in cancer. About 15% of patients with CRC harbour dMMR, and in about 20% of those cases, the cancers are related to Lynch syndrome, which is secondary to germline mutations[67,68]. Approximately 5% of patients with mCRC have dMMR disease, and 34.6% have a *BRAF* V600E mutation, which suggests sporadic dMMR instead of Lynch syndrome[69].

Immune checkpoints are key regulators of the immune system. Checkpoint proteins expressed by various cells, including tumour cells, block active immune surveillance. Checkpoint inhibitors restore immune system function by targeting those proteins.

In a phase II study, 28 patients with mCRC were treated with pembrolizumab, a humanized PD-1 monoclonal antibody[70]. In the 10 patients with dMMR, the response rate was 40% and the disease control rate was 90%. In contrast, no response was observed in patients with proficient MMR mCRC. The U.S. FDA approved pembrolizumab after a fluoropyrimidine, oxaliplatin, and irinotecan for patients with dMMR mCRC.

The open-label phase II CheckMate 142 study had 6 experimental arms[71]. In the nivolumab (a PD-1 monoclonal antibody) monotherapy arm, 74 patients with dMMR mCRC who had received at least 1 line of treatment were enrolled. The investigator-assessed ORR was 31.1% (23 of 74 patients), and 69% of the patients ($n = 51$) experienced disease control for 12 or more weeks[71]. In the nivolumab–ipilimumab (a CTLA-4 antibody) combination arm, 119 patients with dMMR mCRC were treated for 4 cycles and then received nivolumab as a single agent every 2 weeks until disease progression[72]. The ORR was 55%, with 80% of patients achieving disease control for 12 or more weeks. The PFS was 76% at 9 months and 71% at 12 months. Nivolumab was also approved by the U.S. FDA for the treatment of dMMR mCRC after chemotherapy with or without ipilimumab.

BRAF *V600E Mutated mCRC:* In the EGFR/RAS/RAF/MEK/ERK pathway, BRAF is a key player. The *BRAF* V600E mutation ($BRAF^{V600E}$) causes constitutive activation of MAPK signalling. Approximately 8% of patients with CRC harbour $BRAF^{V600E}$, which has distinct clinicopathologic characteristics[73,74]. Cases of $BRAF^{V600E}$ mCRC are more commonly seen in older female patients and typically have poor clinical outcomes[20,75,76].

Approximately 20% of patients with $BRAF^{V600E}$ mCRC also have dMMR or MSI-H, and immune checkpoint inhibitor treatments have proved to be effective for those patients[71,72]. However, most patients with $BRAF^{V600E}$ mCRC have proficient MMR, and their disease progresses rapidly with standard chemotherapy. Intensified chemotherapy with FOLFOXIRI–bevacizumab seems to be beneficial in this subset of patients based on an analysis of the TRIBE study[77]. Of 28 patients with $BRAF^{V600E}$ mCRC in the TRIBE study, median OS was better in the FOLFOXIRI–bevacizumab arm (16 patients) than in the FOLFIRI–bevacizumab arm (19.0 months vs. 10.7 months).

It was learned from early studies that a BRAF inhibitor does not have significant single-agent activity in $BRAF^{V600E}$ mCRC, which might be attributable to the feedback activation of EGFR signalling[78–80]. Combining an EGFR inhibitor with a BRAF inhibitor was subsequently tested in the SWOG 1406 study, which enrolled 106 patients with $BRAF^{V600E}$ extended-*KRAS* wild-type mCRC. Patients were treated with irinotecan–cetuximab with or without vemurafenib (a BRAF inhibitor)[81]. A better mPFS (primary endpoint, 4.4 months vs. 2.0 months), ORR (16% vs. 4%), and disease control rate

(67% vs. 22%) were observed in the vemurafenib arm compared with the no-vemurafenib arm.

Given the critical role of downstream MAPK signalling in *BRAF*^{V600E} mCRC and the successes observed from the combination of a BRAF inhibitor with a MEK inhibitor in melanoma treatment[82,83], a similar approach was also tested in *BRAF*^{V600E} mCRC. In a phase I/II clinical trial, triplet treatment with dabrafenib (a BRAF inhibitor), trametinib (a MEK inhibitor), and panitumumab was associated with a higher response rate than was seen with panitumumab–dabrafenib (21% vs. 10%). No response was detected in the panitumumab–trametinib arm[84].

The randomized open-label phase III BEACON trial was designed to further test the efficacy of a novel triplet treatment [binimetinib (a MEK inhibitor), encorafenib (a BRAF inhibitor), and cetuximab] for previously treated *BRAF*^{V600E} mCRC[85]. The study had a safety lead-in phase in which 30 patients were enrolled and treated with that triplet. The data from those 30 patients confirmed an ORR of 48%, with a mPFS of 8.0 months and a mOS of 15.3 months, all of which were significantly better than results in historical controls. The preliminary result from the phase III study again confirmed an improved mOS (9.0 months vs. 5.4 months) and ORR (26% vs. 2%) when the triplet was compared with irinotecan–cetuximab treatment[35]. The triplet treatment is now considered to be the new standard treatment for *BRAF*^{V600E} mCRC.

HER2-*Positive mCRC:* The HER2 member of the human EGFR family has been a successful treatment target in breast cancer, esophageal adenocarcinoma, and gastroesophageal junction adenocarcinoma. Overexpression of HER2 in CRC is rare, but its prevalence is higher (5%–14%) in *KRAS, NRAS,* and *BRAF* wild-type mCRC[86,87].

The Italian open-label phase II HERACLES study enrolled 27 patients with *KRAS* exon 2 wild-type *HER2*-positive mCRC[86]. The patients were heavily treated before study enrolment, having received an average of 4 previous lines of treatment. Treatment with trastuzumab (a HER2 antibody) and lapatinib (a HER2 tyrosine kinase inhibitor) was associated with an ORR of 30% (8 patients), with 1 patient experiencing a complete response and 12 patients (44%) having stable disease. Recently, the MyPathway study reported on dual HER2 antibody inhibition with pertuzumab and trastuzumab in 57 patients with HER2-overexpressing mCRC[88]. The ORR was 32% (18 patients), with 1 patient experiencing a complete response. Most of the patients were *KRAS* wild-type (75%) and had received multiple previous lines of treatment.

Given the encouraging results from the foregoing studies, the U.S. National Comprehensive Cancer Network guideline lists HER2-targeted treatment as an option for HER2-overexpressing mCRC.

NTRK *Fusion mCRC:* The NTRK family consists of 3 members that are encoded by the *NTRK1, NTRK2,* and *NTRK3* genes. Gene fusions involving those genes lead to constitutively activated NTRK protein and tumorigenesis[89]. Colorectal cancer is one of the first cancer types in which a *NTRK* gene translocation (*TPM3–NTRK1*) was detected (in 1986)[90], but *NTRK*-fusion CRC did not capture a lot of attention until recently, when novel therapies targeting the associated pathway showed remarkable antitumour activity[91]. The small subset of CRCs that harbour a *NTRK* fusion gene (0.1%–1%) might benefit from larotrectinib, a highly selective small-molecule pan-*NTRK* inhibitor[92,93]. An early-phase study of larotrectinib treatment enrolled 55 patients with 17 unique *NTRK* fusion–positive cancers (including 4 colon cancers)[94]. In that population, the *NTRK* fusions involved all 3 family members, and 14 unique upstream fusion partners were detected. Larotrectinib was associated with an ORR of 75% by independent radiology review. Most of the responses were durable (median duration of response was not reached at a median follow-up of 8.3 months), and 71% of responses were ongoing at 1 year. Given the significant clinical benefits, larotrectinib is approved by the U.S. FDA for the treatment of *NTRK* fusion–positive solid tumours and has been incorporated into the CRC treatment guideline from the U.S. National Comprehensive Cancer Network.

SUMMARY

Worldwide, mCRC is a lethal disease, although the mortality rate is improving with recent advancements in treatment. In the first and subsequent lines of treatment, biologics play a very important role. The choice of biologics and their sequencing depend largely on patient factors (preferences, age, performance status, etc.) and disease characteristics [disease sidedness; *KRAS, NRAS,* and *BRAF* mutation status; MSI status; HER2 overexpression, etc. (Figure 1)]. To

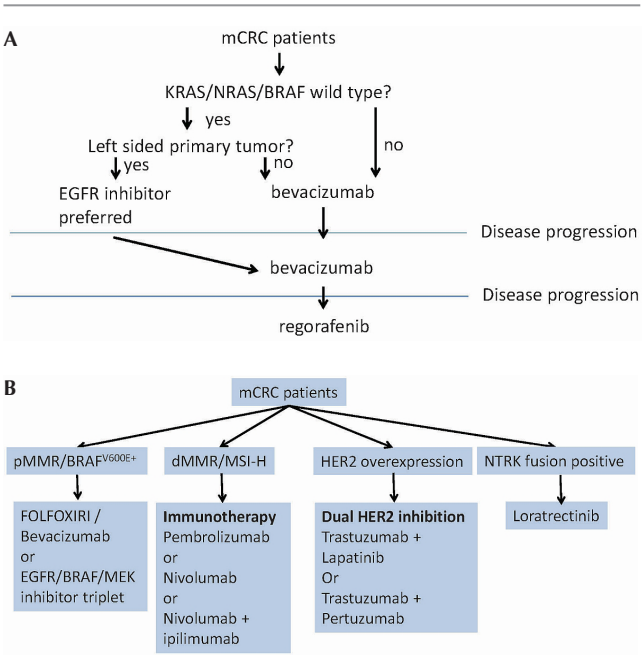

FIGURE 1 (A) Simplified consideration for choosing biologics. (B) Biologics for specific patient populations.
mCRC = metastatic colorectal cancer; EGFR = epidermal growth factor receptor; pMMR = proficient mismatch repair; dMMR = deficient mismatch repair; MSI-H = high microsatellite instability; HER2 = human epidermal growth factor receptor 2; FOLFOXIRI = fluorouracil–leucovorin–oxaliplatin–irinotecan.

maximize survival benefits, it is crucial that patients with mCRC are exposed to all potentially active medications during the course of their treatment.

Although most patients with mCRC will start with a doublet chemotherapy backbone plus a biologic, young and symptomatic patients could benefit from upfront FOLFOXIRI–bevacizumab. Fluoropyrimidine–bevacizumab might be more appropriate for frail patients. Data support the use of first-line EGFR inhibitors for left-sided mCRC that is *KRAS, NRAS,* and *BRAF* wild-type; bevacizumab can be used for both left- and right-sided mCRC. Continuation of bevacizumab, but not EGFR inhibitors, has demonstrated a modest survival benefit after first-line disease progression. The EGFR inhibitors have single-agent activity; bevacizumab lacks data to support its use as a single agent for later lines of treatment. Immune checkpoint inhibitors are critical for the treatment of patients with dMMR (MSI-H) mCRC in both Lynch syndrome and sporadic mutation cases. The novel triplet combination of an EGFR inhibitor, a MEK inhibitor, and a BRAF inhibitor should be incorporated into daily practice for $BRAF^{V600E}$ mCRC. Dual HER2 inhibition with trastuzumab–lapatinib or trastuzumab–pertuzumab for HER2-overexpressing mCRC has some very promising preliminary data, and ongoing studies will likely lead to regulatory approval of that strategy.

The advancement of cancer treatments in CRC is based on a better understanding of tumour biology and the tumour microenvironment. Molecular analysis of each patient's tumour by next-generation sequencing upon diagnosis is recommended to determine the optimal systemic therapy combinations throughout the course of the patient's disease. That approach will also identify additional mutations or alterations and might identify clinical trial opportunities involving the next generation of biologic therapies.

CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare the following interests: JMH has served as an advisory board member for Bayer Pharmaceuticals and her institution receives funding from Merck, Taiho Oncology, and Bayer Pharmaceuticals for trials in which she is an investigator. ZJ has no conflicts of interest to disclose.

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