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REVIEW ARTICLE

Targeting Angiogenesis in Colorectal Carcinoma

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Abstract
Neo-angiogenesis plays a key role in colorectal cancer, with the vascular endothelial growth factor family proteins and their receptors in particular triggering multiple signaling networks that result in endothelial cell survival, migration, mitogenesis, differentiation, and vascular permeability. Anti-angiogenic therapies have improved colorectal cancer prognosis within the past 15 years. Bevacizumab demonstrated efficacy in combination with chemotherapy under different conditions, including as first- and second-line therapies, and also as a maintenance treatment strategy. Other drugs targeting angiogenesis effectors (e.g., ramucirumab and aflibercept) were approved after bevacizumab failure, confirming the concept of "continuous anti-angiogenic blocking". Recently, a number of new orally available multiple receptor tyrosine kinase inhibitors have been tested in late-stage clinical trials, with modest efficacy. Due to the availability of several anti-angiogenic agents, we need well-designed prospective randomized trials to optimize therapeutic sequencing. The place of biosimilars in the therapeutic armamentarium remains unclear at the moment. Further research is warranted to identify robust predictive biomarkers of efficacy and innovative clinically meaningful anti-angiogenic drugs that are cost-efficient.

Key Points

Anti-angiogenic therapies have significantly improved metastatic colorectal cancer prognosis, demonstrating efficacy in combination with chemotherapy under different conditions (e.g., first- and second-line settings, maintenance strategy).

Recently, several orally available multiple receptor tyrosine kinase inhibitors have been tested in later stages of the disease, with modest efficacy.

Current ways of research encompass the identification of robust predictive biomarkers of efficacy and the possibility of substituting biosimilars for original drugs.

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1 Introduction

Incidence rates of colorectal cancer (CRC) have been falling on average by 2.7% each year over the last decade [1], but it is estimated that almost 135,500 new cases were diagnosed in the USA in 2017 [2]. Although commonly associated with good outcomes, 5-year overall survival (OS) is about 65% across all stages, and decreases to 15% in case of distant metastases [2]. Despite strong hereditary components, extrinsic factors such as physical activity, sedentary behavior, and diet seem to be key factors in colorectal carcinogenesis [3].

At a molecular level, most CRCs (85%) show a microsatellite stable (MSS) or low-level microsatellite instability (MSI-L) phenotype, and are characterized by chromosomal changes, leading to the classic adenoma–carcinoma pathway [4]. About 15% of colorectal tumors have a high-level microsatellite instability (MSI-H) phenotype as a result of DNA mismatch repair deficiency. Among MSI-H tumors, 3% are related to Lynch syndrome and 12% correspond to sporadic tumors [4]. There is increasing evidence demonstrating a relationship between molecular pathogenesis, prognosis, and therapy response. A more refined classification based on gene expression was recently developed thanks to an international consortium of experts, defined as the "consensus molecular subtypes" (CMSs) of CRC [5].

Four subtypes were thus identified: CMS1 (microsatellite instability immune), CMS2 (canonical), CMS3 (metabolic), and CMS4 (mesenchymal). The latter represents almost one-quarter of the CRCs, and is characterized by high expression of mesenchymal genes, stromal infiltration, transforming growth factor beta (TGF-β) activation, and angiogenesis. CMS4 tumors display worse overall and relapse-free survival compared with other molecular subtypes, underlying the essential role of angiogenesis in CRC progression [5]. However, in the pan-GI TCGA analysis, the CMS subtypes did not map well, casting some doubt regarding their long-term utility [6]. Additionally, a significant proportion of CRC tumors do not classify in the CMS categories.

When neo-angiogenesis is not possible for several reasons, cancer stem cells are able to reprogram themselves to form blood vessels as a backup strategy. Activation of the transforming growth factor beta (TGF-β) pathway thus seems to play a key role in the initiation of angiogenesis in colon cancer stem cells [7], opening the way to potential novel therapeutic approaches.

The relationship between neo-angiogenesis and tumor proliferation was first described almost 50 years ago [8], but the clinical efficacy of bevacizumab [a monoclonal antibody against vascular endothelial growth factor (VEGF)] in CRC patients was only demonstrated in 2004 [9]. Several growth factor receptor pathways have been implied to promote tumor angiogenesis, but the VEGF family proteins and their receptors play a central role, triggering multiple signaling networks that result in endothelial cell survival, migration, mitogenesis, differentiation, and vascular permeability [10]. We currently know of six effectors in the VEGF family secreted by tumor cells (VEGF-A to D and placental growth factor (PIGF) 1 and 2), binding to three different types of receptors (VEGFR-1, VEGFR-2, and VEGFR-3). The interaction between VEGF and its receptor leads to the dimerization of two receptors. Subsequently, the tyrosine kinase domain of each receptor phosphorylates the other, which initiates a signaling cascade involving the activation of several pathways such as Ras-Raf-MAPK, Scr-FAK, or AKT-mTOR. The VEGF pathway is upregulated by several growth factors, including epidermal growth factor (EGF), platelet-derived growth factors (PDGFs), hepatocyte growth factor (HGF), and other cytokines [11]. The presence of elevated circulating levels of VEGF has been shown to be predictive of liver and lung metastasis [12]. High VEGF serum level was associated with poorer survival in case of CRC. In a meta-analysis published in 2014, hazard ratio (HR) for death was 2.25 (95% confidence interval (CI) 1.35–3.74) [13].

Tumor angiogenesis can be blocked through several ways. Anti-angiogenic monoclonal antibodies bind to and therefore neutralize a specific target such as VEGF-A (bevacizumab) or VEGFR-2 (ramucirumab). Aflibercept is a recombinant fusion protein inhibiting the VEGF-A, VEGF-B, and PIGF pathways, which may help to overcome tumor escape mechanisms to bevacizumab treatment. Novel antiangiogenic agents, essentially oral tyrosine kinase inhibitors (TKI), seem promising in targeting several signaling pathways, even in heavily pretreated CRC patients. In this review, we discuss and highlight current and future approaches in angiogenic targeting for CRC.

2 Bevacizumab

2.1 First-Line Treatment

Bevacizumab is a recombinant humanized (93% human and 7% murine) monoclonal immunoglobulin G1 antibody that binds to all isoforms of VEGF-A (Fig. 1) with a reported half-life of 17–21 days, preventing the interaction between VEGF-A and VEGFR-1 and -2 [14]. AVASTIN® (Roche) was the first anti-VEGF agent approved as first-line therapy for metastatic CRC (mCRC) patients, In the first-line setting, bevacizumab was associated with an increased median OS compared with placebo in 813 patients with previously untreated mCRC (HR 0.66; $p < 0.001$) [9]. (Table 1) All patients received IFL (irinotecan, bolus fluorouracil, and leucovorin) as backbone chemotherapy, but capecitabine plus oxaliplatin (XELOX) or fluorouracil/folinic acid plus oxaliplatin (FOLFOX) were also valuable choices [15]. A recent phase III study confirmed the absence of difference between FOLFIRI (5-fluoruracil, leucovorin, and irinotecan) and FOLFOX in addition to bevacizumab in treatment-naïve mCRC patients [16]. The 2016 ESMO guidelines recommend any chemotherapy doublet with bevacizumab as first-line treatment, especially in patients with a RAS mutated tumor [17]. In a meta-analysis of nine studies and 3710 patients with mCRC, the addition of bevacizumab to chemotherapy significantly prolonged progression-free survival (PFS) (HR 0.66, 95% CI 0.55–0.77; $p < 0.0001$) and OS (HR 0.84, 95% CI 0.77–0.92; $p = 0.0001$) [18].

The concept of intensive chemotherapy associated with high response rates has recently emerged, with the aim of surgery in patients with potentially resectable liver metastases. In the TRIBE study, 508 patients with untreated mCRC received FOLFIRI plus bevacizumab or FOLFOXIRI plus bevacizumab [19]. The objective response rate (ORR) was 65% in the experimental group and 53% in the control group ($p = 0.006$). The median PFS was significantly increased (HR 0.75, 95% CI 0.62–0.90; $p = 0.003$), but incidences of grade 3 or 4 neurotoxicity, stomatitis, diarrhea, and neutropenia were significantly higher in cases of triplet chemotherapy (Table 2). These results were confirmed in a randomized phase II trial with FOLFOX as control treatment [20]. In a systematic review with pooled analysis, including

Table 1 Outcomes of major studies evaluating anti-angiogenic therapies in metastatic colorectal cancer patients

Drug	Study	First author, year	NCT number	Line	*N*	Treatment	Comparator	ORR (%)	PFS (mo)	OS (mo)	References
Bevacizumab	–	Hurwitz, 2004	–	1st	813	Bevacizumab + IFL	IFL	44.8	10.6	20.3	[9]
	NO16966	Saltz, 2008	–	1st	1401	Bevacizumab + FOLFOX-4 or XELOX	FOLFOX-4 or XELOX	–	9.4	21.3	[15]
	ITACa	Passardi, 2015	01878422	1st	376	Bevacizumab + FOLFOX or FOLFIRI	FOLFOX or FOLFIRI	50.6	9.6	20.8	[16]
	TRIBE	Loupakis, 2014	00719797	1st	508	Bevacizumab + FOLFOXIRI	Bevacizumab + FOLFIRI	65.1	12.1	31.0	[19]
	OLIVIA	Gruenberger, 2015	00778102	1st	80	Bevacizumab + FOLFOXIRI	Bevacizumab + mFOLFOX-6	81.0	18.6	–	[20]
	AVEX	Cunningham, 2013	00484939	1st	280	Bevacizumab + capecitabine	Capecitabine	74.0	9.1	20.7	[22]
	PRODIGE 20	Aparicio, 2017	01900717	1st	102	Bevacizumab + chemotherapy[a]	Chemotherapy[a] alone	37.2	9.7	21.7	[23]
	CAIRO3	Simkens, 2015	00442637	Maintenance	558	Bevacizumab + capecitabine	Observation	69.0	8.5	21.6	[26]
	AIOI 0207	Hegewisch-Becker, 2015	00973609	Maintenance	472	Bevacizumab + fluoropyrimidine	Bevacizumab or observation	–	6.3	20.2	[27]
	E3200	Giantonio, 2007	00025337	2nd	829	Bevacizumab + FOLFOX4	Bevacizumab or FOLFOX	22.7	7.3	12.9	[30]
	ML18147/TML	Bennouna, 2013	00700102	2nd	409	Bevacizumab + chemotherapy[b]	Chemotherapy[b] alone	5.0	5.7	11.2	[34]
	BEBYP	Masi, 2015	00720512	2nd	185	Bevacizumab + mFOLFOX-6 or FOLFIRI	mFOLFOX-6 or FOLFIRI	21.0	6.8	14.1	[35]
Ramucirumab	RAISE	Tabernero, 2015	01183780	2nd	1072	Ramucirumab + FOLFIRI	FOLFIRI	13.4	5.7	13.3	[38]
Aflibercept	VELOUR	Van Cutsem, 2012	00561470	2nd	1226	Aflibercept + FOLFIRI	FOLFIRI	19.8	6.9	13.5	[43]
Regorafenib	CORRECT	Grothey, 2013	01103323	≥3rd	760	Regorafenib	Placebo	1.0	1.9	6.4	[46]
	CONCUR	Li, 2015	01584830	≥2nd	204	Regorafenib	Placebo	4.0	3.2	8.8	[48]
Nintedanib	LUME-Colon 1	Van Cutsem, 2016	02149108	≥3rd	765	Nintedanib	Placebo	0.0	1.5	6.4	[52]
Fruquintinib	–	Xu, 2017	02196688	≥2nd	42	Fruquintinib	Placebo	2.1	5.8	8.9	[56]

ORR objective response rate, *PFS* progression-free survival, *OS* overall survival

[a]mFOLFOX6 or mFOLFIRI or LV5FU2

[b]FOLFOX or XELOX or FOLFIRI or XELIRI

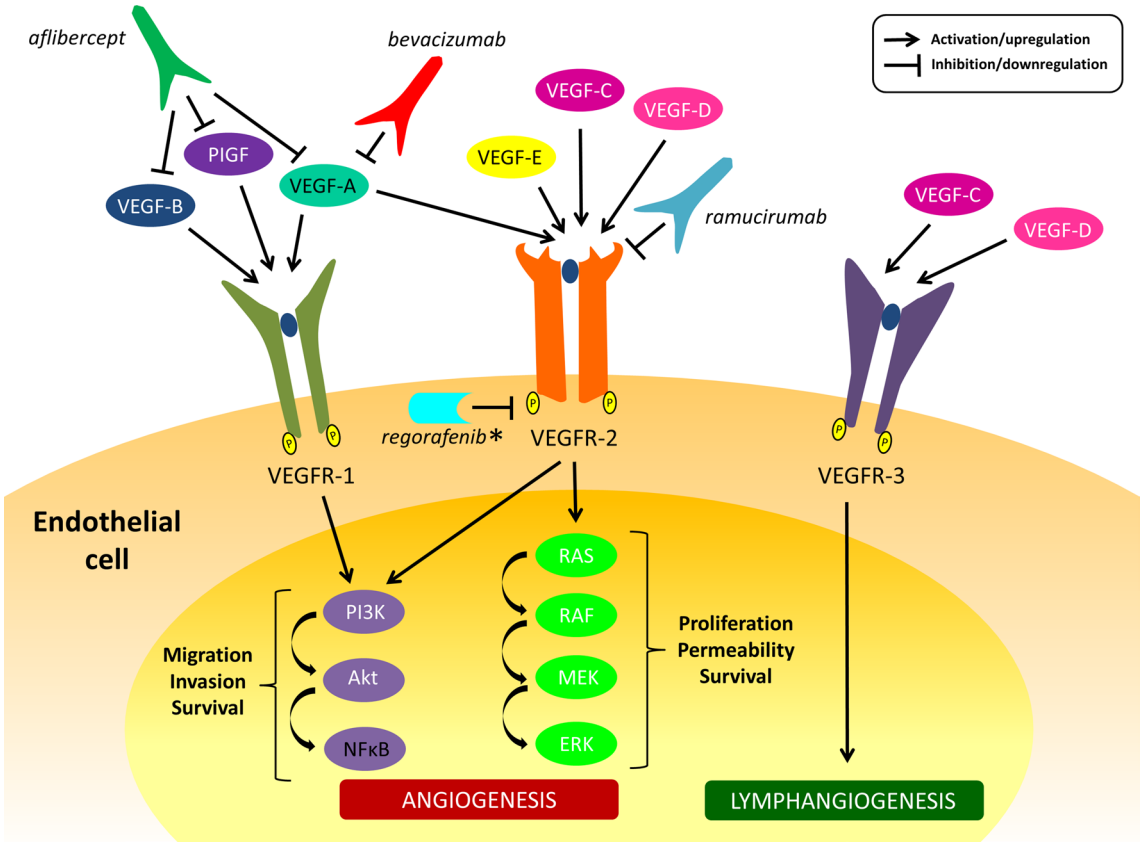

Fig. 1 Vascular endothelial growth factor (VEGF) pathway and targeted therapies. *PIGF* placental growth factor, *VEGFR* vascular endothelial growth factor receptor, *PI3K* phosphoinositide 3-kinase, *Akt* protein kinase B, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *RAS* rat sarcoma, *RAF* rapidly accelerated fibrosarcoma, *MEK* mitogen-activated protein kinase, *ERK* extracellular signal-regulated kinases. *Regorafenib also inhibits multiple targets in addition to VEGFR-2, not shown in this figure: PDGFR, FGF, KIT, RET, RAF1, B-RAF, and B-RAF-V600E

11 FOLFOXIRI-bevacizumab studies ($n = 889$), the ORR was 69% [21]. The rate of overall surgical conversions was 39%, and the rate of R0 surgical conversions was 28%.

Elderly patients are often under-represented in clinical trials although they represent a significant proportion of patients seen at our practices. In the phase III AVEX trial, 280 patients aged 70 years and older who were not deemed to be candidates for oxaliplatin-based or irinotecan-based chemotherapy regimens were randomly assigned to capecitabine alone or with bevacizumab [22]. Median PFS was significantly longer with bevacizumab and capecitabine than with capecitabine alone (9.1 months vs. 5.1 months, HR 0.53, 95% CI 0.41–0.69; $p < 0.0001$). Bevacizumab was generally well tolerated, albeit with more grade 3 or worse treatment-related adverse events compared with placebo (40% vs. 22%), especially hemorrhage (25% vs. 7%), hand-foot syndrome (16% vs. 7%), and venous thromboembolic events (8% vs. 4%). A phase II study recently confirmed the possibility for treating elderly patients effectively and safely with chemotherapy doublets and bevacizumab in the first-line setting [23].

Finally, in the adjuvant setting the addition of bevacizumab to the standard FOLFOX regimen failed to improve disease-free survival in two large phase III studies conducted in stage II and III CRC patients [24, 25].

2.2 Maintenance Treatment

The optimum duration of first-line treatment for mCRC is complex. On the one hand, the longer the chemotherapy duration is, the higher the cumulative toxicity is, with potential impaired quality of life and increasing treatment cost. On the other hand, longer duration of treatment is associated with a longer PFS, and potentially increases OS. In the phase III CAIRO3 study, 558 patients with previously untreated mCRC and stable disease or better after induction treatment with six 3-weekly cycles of capecitabine, oxaliplatin, and bevacizumab (CAPOX-B) were randomly assigned to either maintenance treatment with capecitabine and bevacizumab (CAP-B) or observation [26]. Median PFS between randomization and first progression was significantly improved in case of maintenance therapy compared

Table 2 Safety profile of major anti-angiogenic therapies in metastatic colorectal cancer patients

Drug	Study	First author, year	Associated treatment	Grade 3–4 adverse events (%)										Death	References
				Any	HT	TE	Bleeding	Proteinuria	Neutropenia	FN	Diarrhea	HFS	GI perforation		
Bevacizumab	–	Hurwitz, 2004	IFL	84.9	11.0	19.4	3.1	0.8	37.0	–	32.4	–	1.5	2.6	[9]
	NO16966	Saltz, 2008	FOLFOX-4 or XELOX	80.0	4.0	10.0	2.0	< 1.0	–	–	–	–	< 1.0	2.0	[15]
	ITACa	Passardi, 2015	FOLFIRI or FOLFOX4	–	27.8[a]	21.0	4.6[a]	13.4[a]	39.6	0.6	5.7	–	–	2.3	[16]
	TRIBE	Loupakis, 2014	FOLFOXIRI	–	5.2	7.2	–	–	50.0	8.8	18.8	–	–	2.4	[19]
	OLIVIA	Gruenberger, 2015	FOLFOXIRI	95.0	0.0	8.0	8.0	0.0	50.0	13.0	30.0	–	8.0	0.0	[20]
	AVEX	Cunningham, 2013	Capecitabine	60.0	2.0	9.0	0.0	1.0	1.0	–	1.0	0.0	0.0	4.0	[22]
	PRODIGE 20	Aparicio, 2017	Chemotherapy[b]	82.4	13.7	9.8	2.0	–	11.8	0.0	9.8	–	2.0	–	[23]
	CAIRO3	Simkens, 2015	Capecitabine	60.0	24.0	–	–	–	–	–	–	23.0	–	–	[26]
	AIOI 0207	Hegewisch-Becker, 2015	Fluoropyrimidine	–	3.0	2.0	–	0.0	2.0	–	2.0	3.0	1.0	< 1.0	[27]
	E3200	Giantonio, 2007	FOLFOX4	75.3	6.2	3.4	3.4	0.7	–	–	–	–	1.0	1.7	[30]
	ML18147/TML	Bennouna, 2013	Chemotherapy[c]	–	2.0	5.0	2.0	–	16.0	–	10.0	–	2.0	1.0	[34]
	BEBYP	Masi, 2015	mFOLFOX-6 or FOLFIRI	43.0	2.0	2.0	0.0	5.0	26.0	5.0	7.0	–	0.0	1.1	[35]
Ramucirumab	RAISE	Tabernero, 2015	FOLFIRI	79.0	12.0	6.0	3.0	4.0	10.0	1.0	1.0	0.0	1.0	4.0	[38]
Aflibercept	VELOUR	Van Cutsem, 2012	FOLFIRI	83.4	19.3	9.6	3.0	7.8	36.7	1.3	19.3	2.8	0.5	–	[43]
Regorafenib	CORRECT	Grothey, 2013	Regorafenib	54.0	7.0	0.0	0.0	1.0	0.0	0.0	8.0	17.0	0.0	2.0	[46]
	CONCUR	Li, 2015	Regorafenib	53.0	11.0	0.0	0.0	1.0	2.0	0.0	1.0	16.0	0.7	1.0	[48]
Nintedanib	LUME-Colon 1	Van Cutsem, 2016	Nintedanib	41.9	4.7	0.0	0.3	0.5	0.0	0.0	2.6	0.0	0.0	1.6	[52]
Fruquintinib	–	Xu, 2017	Fruquintinib	–	29.8	< 4.0	< 4.0	< 4.0	< 4.0	< 4.0	2.1	14.9	< 4.0	2.1	[56]

HT hypertension, *TE* thrombotic event, *FN* febrile neutropenia, *HFS* hand-foot syndrome

[a] Any grade

[b] mFOLFOX6 or mFOLFIRI or LV5FU2

[c] FOLFOX or XELOX or FOLFIRI or XELIRI

with observation (8.5 months vs. 4.1 months). The AIO 0207 study was a non-inferiority randomized phase III trial comparing standard maintenance treatment with a fluoropyrimidine plus bevacizumab, bevacizumab alone, or no treatment in 472 mCRC patients without disease progression after 24 weeks of induction therapy with either fluorouracil plus leucovorin plus oxaliplatin or capecitabine plus oxaliplatin, both with bevacizumab [27]. Median time to failure of strategy was 6.9 months for the fluoropyrimidine plus bevacizumab group, 6.1 months for the bevacizumab-alone group, and 6.4 months for the no-treatment group. In a post hoc analysis, CAP-B maintenance treatment after six cycles of CAPOX-B is effective in mCRC across all mutational subgroups (RAS wild-type/RAS mutant, BRAF wild-type/BRAF mutant, MSS/MSI tumors) [28]. A recent meta-analysis of these two phase III trials confirmed that maintenance treatment with fluoropyrimidine plus bevacizumab is effective in all patients, regardless of the investigated subgroups (sex, age, performance status, response to induction treatment, primary tumor location, number of metastatic sites, disease stage and primary tumor resection, serum LDH, platelet count, CEA, and RAS/BRAF mutation status) [29]. The 2016 ESMO guidelines recommend that patients receiving FOLFOX or CAPOX plus bevacizumab-based therapy as induction therapy should be considered for maintenance therapy after six cycles of CAPOX and eight cycles of FOLFOX [17]. The optimal maintenance treatment is therefore a combination of a fluoropyrimidine plus bevacizumab, whereas bevacizumab as monotherapy is not recommended.

2.3 Second-Line Treatment

In bevacizumab-naïve mCRC patients previously treated with FOLFIRI, the combination of FOLFOX and bevacizumab was associated with a reduced risk of death (HR 0.75; $p=0.0011$) compared with FOLFOX alone [30]. PFS and ORR were also significantly improved. Preclinical data also suggest that VEGF has a continuous expression during tumor progression and that a prolonged exposure to anti-angiogenic agents beyond progression could delay tumor growth [31]. After promising retrospective data [32, 33], several prospective studies confirmed the concept of "continuous anti-angiogenic blocking". In the ML18147/TML study, 409 patients with mCRC progressing up to 3 months after discontinuing first-line bevacizumab plus chemotherapy were randomly assigned to second-line chemotherapy with or without bevacizumab [34]. Median OS was 11.2 months for bevacizumab plus chemotherapy and 9.8 months for chemotherapy alone ($p=0.0062$). A possible resistance mechanism to anti-angiogenic agents may include increased levels of circulating VEGFA levels, able to interact once again with VEGFR. The BEBYP study had a similar design but was prematurely stopped after 185 randomized patients in consideration of the results of the ML18147 trial [35]. The median PFS was 5.0 months in the chemotherapy group and 6.8 months in the bevacizumab group ($p=0.010$). An improved OS was also observed in the bevacizumab arm (adjusted HR = 0.77; 95% CI 0.56–1.06; $p=0.043$). To conclude, the ESMO recommendations state that patients who received bevacizumab first line should be considered for treatment with bevacizumab as a post-discontinuation strategy [17].

3 Ramucirumab

Ramucirumab is a fully human IgG1 monoclonal antibody targeting VEGFR-2, considered as the primary VEGF family receptor driving angiogenesis [36]. As a consequence, the blockade of VEGFR-2 induced by ramucirumab prevents the interaction of all VEGF ligands and receptor activation. After an interesting activity profile and acceptable tolerance were shown in a phase I study [37], ramucirumab was tested in combination with FOLFIRI vs. placebo as second-line treatment in mCRC patients in a large phase III study (RAISE) [38]. Eligible patients had disease progression during or within 6 months of the last dose of first-line combination therapy with bevacizumab and FOLFOX. In the 1072 enrolled patients (536 in each arm), median OS was significantly improved with ramucirumab compared with placebo (13.3 months vs. 11.7 months, HR 0.84, $p=0.0219$). Median PFS was increased in the ramucirumab group (5.7 months vs. 4.5 months, HR 0.79, $p=0.0005$). Interestingly, this advantage persisted in patients with fast-growing tumors (time to progression after start of first-line treatment < 6 months), who were not included in the TML study, in which bevacizumab was evaluated in the same setting as ramucirumab. Main grade 3 or worse adverse events were neutropenia (38% vs. 23%, with febrile neutropenia incidence of 3% vs. 2%), hypertension (11% vs. 3%), diarrhea (11% vs. 10%), and fatigue (12% vs. 8%). In a recent meta-analysis including 4996 patients treated with ramucirumab, arterial/venous thromboembolic events and high-grade bleeding were not significantly increased compared with placebo [39]. On the other hand, a higher percentage of hypertension, proteinuria, low-grade bleeding, GI perforation, infusion-related reaction, and wound-healing complications were confirmed in the ramucirumab group.

Contrary to the RAS status with anti-EGFR antibodies, robust predictive biomarkers of efficacy are lacking with anti-angiogenic therapies. Recently, the RAISE biomarker program post hoc analysis found that the median OS in the ramucirumab arm compared with the placebo arm showed an improvement of 2.4 months in the high VEGF-D subgroup (13.9 months vs. 11.5 months, respectively) [40]. PFS results were consistent with OS, but no trends were

evident with the other antiangiogenic candidate biomarkers (VEGF-C, sVEGFR-1, sVEGFR-2, sVEGFR-3 in plasma, and VEGFR-2 in tumor tissue). The RAISE trial supports the hypothesis that inhibition of tumor angiogenesis beyond initial disease progression is an effective treatment strategy. However, in a phase II randomized study, combining ramucirumab or icrucumab (anti-VEGFR-1) with FOLFOX did not achieve the predetermined improvement in PFS in patients with mCRC after disease progression on first-line therapy with FOLFIRI [41]. The ESMO guidelines consider ramucirumab in combination with FOLFIRI as a second-line treatment in patients who received bevacizumab first line, especially in those with fast-growing tumors [17].

4 Aflibercept

Aflibercept is an anti-angiogenic agent and its mechanism of action consists of binding to the endogenous circulating VEGF molecules and to the placental growth factor (PIGF). It is a recombinant fusion protein of the VEGF-binding parts of the extracellular domains of human VEGF receptors 1 and 2, and the Fc portion of the human IgG1 immunoglobulin. It therefore inhibits the activity of VEGF A and B and the formation of new blood vessels within the tumor [42]. By inhibiting the formation of new blood vessels, tumor growth and proliferation are compromised as a result of nutrient deprivation, and its proliferation and invasion is halted.

Aflibercept was approved for the treatment of metastatic colorectal cancer based on the VELOUR study, a phase III randomized double-blind placebo-controlled global multicenter trial, in patients who were resistant to or had progressed following an oxaliplatin-containing regimen, with or without prior bevacizumab [43]. In this trial, 1226 patients were randomized at a 1:1 ratio to receive either aflibercept 4 mg/kg intravenously or placebo, in combination with FOLFIRI. Patients were treated until disease progression or unacceptable toxicity, primary endpoint was OS and secondary endpoints were PFS and ORR. The addition of aflibercept to FOLFIRI significantly improved OS as compared to placebo and the median OS was 13.5 months vs. 12.1 months (HR 0.82, 95% CI 0.71–0.94, $p = 0.003$). PFS was also significantly improved (HR 0.76, 95% CI 0.66–0.87, $p < 0.001$). The improvement of survival was consistent across subgroups, including bevacizumab-pretreated patients. Aflibercept was generally well tolerated and the reported adverse effects were the usual characteristic ones related to other anti-VEGF agents as well as an increase in some chemotherapy-related toxicities.

There are currently several, mostly phase II, clinical trials evaluating the effect of aflibercept first line in locally advanced or mCRC in different combinations with chemotherapy ongoing, among which are two trials with FOLFIRI (NCT02181556 and NCT02624726), one with LV5FU2 (NCT02384759), and another with oxaliplatine/fluoropyrimidine combinations (NCT01802684). There is also a phase II trial evaluating the impact of a personalized marker-driven (based on a cytokines/angiogenic factor profile) treatment approach using aflibercept with FOLFOX after first-line treatment with FOLFOX-bevacizumab (NCT02331927), a phase I study of aflibercept in combination with pembrolizumab for advanced solid tumors (NCT02298959), and two phase II trials with aflibercept in combination with FOLFOX for advanced rectal cancer (NCT02340949, NCT03043729).

5 Regorafenib

Regorafenib is an orally bioavailable multikinase inhibitor targeting several different protein kinases that are involved in important steps of tumor growth and proliferation. Its anti-angiogenic activity is due to its dual targeted VEGFR2-TIE2 tyrosine kinase inhibition. The agent also targets oncogenic factors (KIT, RET, RAF1, B-RAF, and B-RAF-V600E) and acts on the tumor microenvironment and stroma by targeting platelet-derived growth factor receptor and fibroblast growth factor [44]. Regorafenib has demonstrated anti-angiogenic, anti-proliferative, and pro-apoptotic effects in patient-derived murine models of gastric cancer [45].

In the phase III randomized placebo-controlled global multicenter trial (CORRECT), 760 patients with mCRC were randomized to receive oral regorafenib or placebo plus best supportive care [46]. All patients should have progressed within 3 months after several lines of standard treatments, including chemotherapy, bevacizumab, cetuximab, or panitumumab. Regorafenib significantly improved OS compared with placebo (6.4 months vs. 5.0 months, HR 0.77, 95% CI 0.64–0.94, $p < 0.005$). PFS was also significantly improved (HR 0.45, 95% CI 0.42–0.58, $p < 0.001$). Interestingly, the survival benefit was observed across all subgroups irrespective of KRAS status. The major adverse events were hand and foot skin reaction, fatigue, diarrhea, hypertension, and rash/desquamation.

Two other phase III studies (CONSIGN and CONCUR) demonstrated a survival benefit. The CONSIGN trial was a large prospective, open-label, single-arm, global multicenter trial conducted at 188 sites in 25 countries for patients with mCRC who progressed after standard therapies [47]. Its primary endpoint was safety. After analysis of data from 2864 patients who received regorafenib, the safety profile was consistent with data from the CORRECT trial. PFS was in the range of that previously reported and comparable across KRAS wild-type and mutant patient groups. CONCUR was another randomized, double-blind, placebo-controlled, phase III trial that compared regorafenib and placebo in Asian patients with previously treated mCRC [48]. Two hundred and forty-three patients

were enrolled, and after a median follow-up of 7.4 months, OS was significantly improved with regorafenib (HR 0.55, 95% CI 0.40–0.77, $p<0.001$). Adverse events were generally consistent with the known safety profile of regorafenib.

When prescribing regorafenib, physicians may have to face toxicity concerns, especially fatigue, hand-foot syndrome, abdominal pain, or hypertension, in heavily pre-treated patients. In the randomized phase II ReDOS study, a weekly dose escalation of regorafenib from 80 mg to 160 mg/day (Arm A: 80 mg for 1 week, escalation to 120 mg at week 2, and final escalation to 160 mg at week 3) was compared with standard dosing (Arm B: 160 mg/day immediately) [49]. The primary endpoint was the proportion of patients who completed two cycles of treatment and initiated the third in Arm A ($n=54$) vs. Arm B ($n=62$). Forty-three percent of patients in Arm A initiated the third cycle vs. only 25% of patients in Arm B (one-sided p value 0.028). Median OS was improved in Arm A vs. Arm B (9.0 months vs. 5.9 months; $p=0.094$), whereas median PFS was similar. Overall rates of grade 3/4 toxicity were more favorable for Arm A vs. Arm B (hand-foot syndrome 15% vs. 16%, hypertension 7% vs. 15%, and fatigue 13% vs. 18%). Multiple quality-of-life parameters were improved in arm A vs. B primarily at week 2 of the first cycle. This dose-escalation strategy with regorafenib must be confirmed in further studies, but it is considered as a new standard for many prescribers.

Recently, a randomized phase II trial (REVERCE) tried to find the optimal treatment sequence in 101 KRAS exon 2 wild-type mCRC patients, after failure of fluoropyrimidine, oxaliplatin, and irinotecan [50]. Patients were randomized to receive sequential treatment with regorafenib followed by cetuximab ± irinotecan (R–C arm) or the reverse sequence (C ± irinotecan followed by R; C–R arm). Bevacizumab had been previously administered in 96% and 98% of patients in R–C and C–R, respectively. Median OS in R–C and C–R were 17.4 and 11.6 months, respectively (stratified log rank, $p=0.0293$), with an HR of 0.61 (95% CI 0.39–0.96). Additional studies are warranted to confirm these results but once again, it could be explained by continuous anti-angiogenic pressure.

There are many ongoing clinical trials with regorafenib, including a phase II trial assessing potential biomarkers (NCT01949194) and a phase II as second-line treatment in RAS-mutant CRC (NCT02619435). Another prospective translational phase II trial is investigating molecular predictors of resistance and response to regorafenib in RAS-mutant mCRC (NCT03010722).

6 Other Small-Molecule Tyrosine Kinase Inhibitors

Newly developed VEGFR inhibitors are being evaluated in several trials. Firstly, nintedanib is an oral agent that inhibits VEGFR 1–3, platelet-derived growth factor receptors (PDGFR α and β), and fibroblast growth factor receptors (FGFR 1–3). Nintedanib was reported to be effective for non-small-cell lung carcinoma, but not for CRC [51, 52]. The LUME-Colon 1 trial randomized 768 mCRC patients after failure of standard therapies into nintedanib plus best supportive care group ($n=386$) and best supportive care group only ($n=382$) [52]. Nintedanib led to statistically significant improvement in PFS; median PFS in the nintedanib group and placebo group were 1.51 months and 1.38 months, respectively (HR = 0.58, $p<0.0001$). However, there was no difference in OS; median OS in nintedanib group and placebo group was 6.44 months and 6.05 months, respectively (HR = 1.01, $p=0.87$). This result suggests that using nintedanib in a clinical setting might be difficult. Secondly, fruquintinib is a highly selective small molecule inhibitor for VEGFR-1–3, and several preclinical studies demonstrated its efficacy [53, 54]. Phase I trials demonstrated safety of fruquintinib, based on which a phase II trial was performed [55–57]. Seventy-one patients treated with more than second-line therapy were randomized to fruquintinib ($n=47$) or placebo ($n=24$) [56]. PFS was significantly improved in the fruquintinib group compared with the placebo group; median PFS in the fruquintinib group and placebo group were 4.73 months and 0.99 months, respectively (HR = 0.30, 95% CI 0.15–0.59, $p<0.001$). The median OS was 7.72 vs. 5.52 months (HR 0.71, 95% CI 0.38–1.34) [56]. Thus, a randomized phase III study with fruquintinib is expected.

7 Conclusions

Over the past 15 years, anti-angiogenic therapies have significantly improved the prognosis of mCRC patients. Bevacizumab clearly demonstrated efficacy in the first-line setting in association with doublet chemotherapy (FOLFOX or FOLFIRI) or capecitabine alone in elderly patients. Bevacizumab was also associated with high ORR when combined with triplet chemotherapy (FOLFOXIRI) in patients with potentially resectable liver metastases. After disease progression with first-line chemotherapy combined with bevacizumab, several prospective studies confirmed the concept of "continuous anti-angiogenic blocking" with bevacizumab, aflibercept, or ramucirumab. This approach may be applied in patients who progressed after two or more lines of standard treatments, as demonstrated with regorafenib, an orally bioavailable multi-kinase inhibitor. Indirect arguments for continuous anti-angiogenic blocking were provided by strategic studies. In the French multicenter, prospective, open randomized PRODIGE 18 trial, wild-type (wt) KRAS mCRC patients who progressed after first-line therapy with bevacizumab and chemotherapy

were randomized to receive bevacizumab or cetuximab in combination with crossover chemotherapy [58]. Continuation beyond progression with bevacizumab was associated with a numerically higher but not statistically significant median PFS and OS compared to cetuximab plus chemotherapy. The Italian phase III COMETS trial also suggested that anti-EGFR therapy would not be the best choice of targeted therapy after failure with chemotherapy plus bevacizumab in wtKRAS mCRC [59]. Results were similar in the randomized phase II SPIRITT study [60]. As a consequence, the ESMO recommendations state that patients who received bevacizumab first line should be considered for treatment with bevacizumab or ramucirumab post-continuation strategy [17].

Another issue concerns the identification of predictive biomarkers of efficacy. Angiogenesis is a continuous and dynamic process, and some pre-clinical and clinical studies showed a shift in balance in cytokines and angiogenic factors after anti-angiogenic exposure. For example, recruitment of basic fibroblast growth factor, HGF, PIGF, stromal-derived factor-1, and macrophage chemoattractant protein-3 is significantly increased in mCRC patients treated with FOLFIRI-bevacizumab in the first-line setting [61]. The rise in alternate pro-angiogenic actors may represent a mechanism of resistance, suggesting that a different tumor angiogenesis inhibitor could be prescribed in the second-line setting. However, robust clinical trials assessing this concept are missing and therefore the decision is essentially based on objective response, toxicity profile, and patients' preference.

Another contemporary issue is the place of biosimilars. Patents protecting bevacizumab in the USA and Europe are expected to expire soon, opening the way to the approval of several biosimilars. The US Food and Drug Administration (FDA) approved the VEGF inhibitor bevacizumab-awwb (MVASI®, Amgen/Allergan) in September 2017 [62], followed by the EMA in January 2018. We can expect better access to bevacizumab at a global level, with lower health costs. After the approval of filgrastim biosimilars in Europe, the average price per treatment-day dropped by 32% [63]. A biosimilar is a biologic that is deemed to be highly similar to a licensed originator product, with no clinically meaningful differences in safety, purity, or potency, following a rigorous comparison exercise. When a potential biosimilar demonstrates a high degree of similarity to the originator, it can be approved for indications initially not studied during the clinical study. This concept, known as extrapolation, was used for bevacizumab because most of the studies were conducted in lung cancer patients [64], although two potential bevacizumab biosimilars (BEVZ92 and BI 695502) are being studied in patients with mCRC (NCT02069704 and NCT02776683, respectively). In a recent survey, almost 50% of physicians reported they "definitely" or "probably" would prescribe a bevacizumab biosimilar if available, underlying potential barriers and required efforts to implement this new therapeutic approach [65].

Finally, orally available multi-receptor TKIs with activity against several angiogenesis effectors are currently intensively tested in mCRC, with disappointing results in most of the cases, except for famitinib, fruquintinib, and nintedanib, which are in later stages of development.

Targeting angiogenesis provided convincing evidence for improving survival in mCRC patients at different moments of their disease journey, but we need further investigations to identify the optimal therapeutic sequence, predictive biomarkers of efficacy, the place of biosimilars, and new clinically meaningful anti-angiogenic drugs.

Compliance with Ethical Standards

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