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TOPIC HIGHLIGHT

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New approaches in angiogenic targeting for colorectal cancer

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Abstract

Colorectal carcinoma (CRC) is one of the leading causes of cancer death worldwide. In the last decade, the addition of irinotecan and oxaliplatin to standard fluorouracil-based chemotherapy regimens have set the new benchmark of survival for patients with metastatic CRC at approximately 20 mo. Despite these advances in the management of CRC, there is a strong medical need for more effective and well-tolerated therapies. The dependence of tumor growth and metastasis on blood vessels makes angiogenesis a rational target for therapy. One of the major pathways involved in this process is the vascular endothelial growth factor (VEGF) and its receptors (VEGFR). In 2004, the first agent targeting angiogenesis, bevacizumab (BV), was approved as an adjunct to first-line cytotoxic treatment of metastatic CRC. The role of BV as part of adjuvant treatment and in combination with other targeted therapies is the subject of ongoing trials. However, BV is associated with an increase in the risk of arterial thromboembolic events, hypertension and gastrointestinal perforations and its use must be cautious. Novel VEGFR TK inhibitors with different ranges of nanomolar potencies, selectivities, and pharmacokinetic properties are entering phase Ⅲ trials for the treatment of cancer. Conversely, one of these novel agents, vatalanib, has been shown not to confer survival benefit in first and second-line treatment of advanced CRC. The basis of these findings is being extensively evaluated. Ongoing and new well-designed trials will define the optimal clinical application of the actual antiangiogenic agents, and, on the other hand, intensive efforts in basic research will identify new agents with different antiangiogenic approaches for the treatment of CRC. In this review we discuss and highlight current and future approaches in angiogenic targeting for CRC.

INTRODUCTION

Colorectal carcinoma (CRC) is one of the leading causes of cancer death worldwide despite progressive improvements in preventive, diagnostic, and therapeutic approaches[1]. Approximately 50 percent of patients who undergo potentially curative surgery alone ultimately relapse and die of metastatic disease[2]. From the late 50 s, 5-fluorouracil (5-FU) was the only drug approved for the treatment of advanced CRC with an overall response rate (RR) and median survival of 10% and 10 mo, respectively[3,4]. This RR was improved to nearly 25% when leucovorin (LV) was used to modulate 5-FU[5]. Recently, irinotecan and oxaliplatin have been added to the armamentarium of agents with activity in CRC. The addition of these two cytotoxic agents to the standard 5-FU/LV-based regimens improves not only RR, but also overall survival (OS) over 5-FU/LV alone, setting the new benchmark of survival for patients with unresectable advanced CRC at around 20 mo[6-10]. Despite these advances in the management of CRC, there is a strong medical need for more effective and well-tolerated therapies and further improvements in survival are anticipated with the introduction of novel targeted therapies both as single agents and in combination. Among them, anti-angiogenesis agents have become a new therapeutic approach in the metastatic setting. In this review we will discuss and highlight current and future approaches in angiogenic targeting for CRC.

ANGIOGENIC TARGETING

The dependence of tumor growth and metastasis on blood vessels makes angiogenesis one of the fundamental hallmarks of cancer[11] and a rational target for[12]. Several growth factor receptor pathways have been implicated in the promotion of tumor angiogenesis. One of the major pathways involved in this process is the vascular endothelial

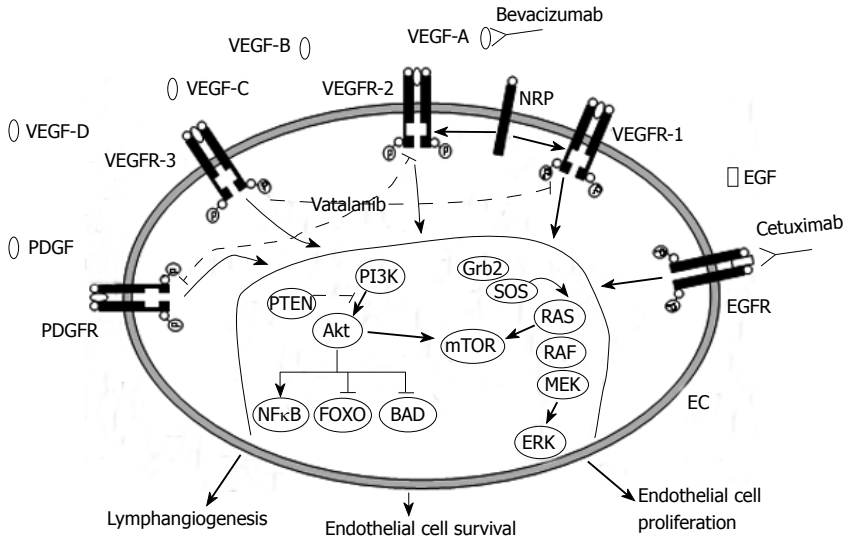

Figure 1 Vascular endothelial growth factor (VEGF) signaling network and novel targeted therapies. VEGFR: Vascular endothelial growth factor receptor; PDGF: Plateled-derived growth factor; PDGFR: Plateled-derived growth factor receptor; EGF: Epidermal growth factor; EGFR: Epidermal growth factor receptor; NRP: Neuropilin; EC: Endothelial cell.

growth factor (VEGF) family of proteins, also known as vascular permeability factors, and its receptors (Figure 1). The VEGF pathway plays a crucial role in normal and pathologic angiogenesis, triggering multiple signaling networks that result in endothelial cell survival, migration, mitogenesis, differentiation, and vascular permeability[13]. The VEGF-related gene family of angiogenic and lymphangiogenic growth factors comprises six secreted glycoproteins referred to as VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placenta growth factor (PlGF) 1 and 2. The primary effects of VEGF ligands are mediated through binding to the VEGF tyrosine kinase receptors (VEGFR): VEGFR-1, which binds VEGF-A, VEGF-B, and PlGF-1; VEGFR-2, which binds VEGF-A, VEGF-C, VEGF-D, and VEGF-E; and VEGFR-3, which binds VEGF-C and VEGF-D, and its expression is limited to the lymphatic endothelial cells. In addition to these receptors, VEGF interacts with neuropilins, a family of activating coreceptors without an intracellular signaling domain[14,15]. VEGFR-1 and VEGFR-2 have seven extracellular immunoglobulin-like domains, a single transmembrane region and a consensus kinase sequence that is interrupted by a kinase-insert domain[16]. Once bound by VEGF, two receptors dimerize, and the tyrosine kinase domain of each receptor "autophosphorylates" the other, leading to an active receptor that initiates a signaling cascade. The VEGF pathway is upregulated by hypoxia[17] and by several growth factors, such as epidermal growth factor (EGF)[18], platelet-derived growth factors (PDGFs)[19,20], hepatocyte growth factor[21] and other cytokines.

Overexpression of VEGF has been associated with tumor progression and poor prognosis in several tumor systems, including CRC[22,23]. Preoperative serum VEGF have also been shown to correlate with advanced tumor stage or nodal status at the time of surgery[24]. Furthermore, intense expression of VEGF mRNA is detected in human liver metastases from primary colon or rectal carcinomas[25]. In 1993, Kim *et al*[26] reported that antibodies to VEGF exert a potent inhibitory effect on the growth of several tumor cell lines in nude mice. In addition, the combination of anti-VEGF antibody and chemotherapy in nude mice injected with human cancer xenografts has an increased antitumor effect compared with antibody or chemotherapy treatment alone[27]. It is, therefore, not surprising that most of the antiangiogenesis treatment strategies focus on inhibition of the VEGF pathway and its regulators. However, the mechanisms of action of anti-VEGF therapy in cancer patients are still far from being fully understood.

In December 2004 the first agent targeting angiogenesis, bevacizumab (Avastin®; Genentech, Inc., South San Francisco, CA), was approved to be given intravenously as a combination treatment along with standard chemotherapy drugs for metastatic CRC, increasing RR, progression-free survival (PFS) and overall survival (OS) with limited toxicity[28]. Gradually, many other antiangiogenic agents that target the VEGF pathway are entering the clinic. These novel targeted agents inhibit the VEGF pathway by targeting the VEGF ligand, its receptors or by blocking downstream signaling pathway components. Antiangiogenic agents include antibodies, low-molecular-weight tyrosine kinase (TK) inhibitors, antisense oligonucleotides and aptamers (Table 1).

BEVACIZUMAB IN CRC

Bevacizumab (BV) is a recombinant humanized monoclonal antibody that binds to all isoforms of VEGF-A with a reported half-life of 17-21 d[29]. In phase I trials, BV was generally well tolerated and did not demonstrate dose-limiting toxicity or interactions with commonly used chemotherapy regimens[30,31]. Based on the data obtained in these phase I trials, Kabbinavar *et al*[32] conducted a randomized, phase II trial comparing the safety and efficacy of BV (at two dose levels, 5 and 10 mg/kg every 2 wk) plus 5-FU (500 mg/m^2)/LV (500 mg/m^2) versus 5-FU/LV alone as first-line therapy for metastatic CRC(Table 2). One hundred and two patients were included. Administration of BV at low-dose and high-dose every 2 wk resulted in a significant increase of 3.8 mo and 2.0 mo, respectively, in the estimated progression-free survival (PFS) compared with 5-FU/LV alone. Treatment with 5-FU/LV/BV at both dose levels compared with 5-FU/LV resulted in

Table 1 Anti-VEGF agents currently in clinical development

Agent	Targets	Phase of development
Specific anti-VEGF antibodies		
Bevacizumab (Avastin)	VEGF-A	Phase Ⅲ
IMC-C1121b	VEGFR-2	Phase Ⅰ-Ⅱ
VEGF Trap	VEGF, PlGF, VEGF-B	Phase Ⅰ
Agents that target VEGF receptors tyrosine kinase		
Vatalanib (PTK787/ZK 222584)	VEGFR1, VEGFR2, VEGFR3, PDGFR-β, c-Kit	Phase Ⅲ
Sorafenib (BAY 43-9006)	VEGFR-2, PDGFR-β, FLT3, c-Kit, Raf	Phase Ⅲ
Sunitinib (SU11248)	VEGFR2, PDGFR-β, FLT3, c-Kit	Phase Ⅲ
Semaxanib (SU5416)	VEGFR1, VEGFR2	Stopped
AZD2171	VEGFR1, VEGFR2, VEGFR3, PDGFR-β, c-Kit	Phase Ⅰ-Ⅱ
CEP-7055	VEGFR1, VEGFR2, VEGFR3	Phase Ⅰ-Ⅱ
CHIR258	VEGFR1, VEGFR2, FGFR1, FGFR3	
CP-547632	VEGFR2	Phase Ⅰ-Ⅱ
GW786034	VEGFR2	Phase Ⅰ-Ⅱ
OSI-930	VEGFR, c-Kit	Phase Ⅰ-Ⅱ
ZK-CDK	VEGFRs, PDGFR, CDKs	Phase Ⅰ-Ⅱ
AG013736	VEGFR, PDGFR-β, c-Kit	Phase Ⅰ-Ⅱ
AMG706	VEGFR1, VEGFR2, PDGFR-β, c-Kit	Phase Ⅰ-Ⅱ
KRN-951	VEGFR1, VEGFR2, VEGFR3, PDGFR-β, c-Kit	Phase Ⅰ-Ⅱ
BMS-582664	VEGFR2, FGFR	Phase Ⅰ-Ⅱ
XL999	FGFR, VEGFRs, PDGFR, FLT3	Phase Ⅰ-Ⅱ
Zactima (ZD6474)	VEGFR2, EGFR, RET	Phase Ⅰ-Ⅱ
AEE788	VEGFR1, VEGFR2, EGFR	Phase Ⅰ-Ⅱ
Antisense oligonucleotides		
Veglin (VEGF-AS)	VEGF, VEGF-C, VEGF-D	Phase Ⅰ
Aptamer		
Aplidin (Dehydrodidemnin B)	VEGF	Phase Ⅰ

CDK: Cyclin-dependent kinase; EGFR: Epidermal growth factor receptor; FGFR: Fibroblast growth factor receptor; FLT3: Fms-related tyrosine kinase 3; MMP: Matrix metalloproteinase; PDGFR: Platelet-derived growth factor receptor; PIGF: Placental growth factor; VEGF: Vascular endothelial growth factor.

Table 2 Completed trials for Bevacizumab with chemotherapy in metastatic CRC

REF	Regimen	Pts	RR (%)	*P*	PFS or TTP (mo)	*P*	OS (mo)	*P*
28	IFL	411	35	0.004	6.2	< 0.001	15.6	< 0.001
	IFL + BV	402	45		10.6		20.3	
32	5-FU/LV	35	17	-	5.2	-	13.6	-
	5-FU/LV + BV-low	35	40	0.029	9	0.005	21.5	0.137
	5-FU/LV + BV-high	32	24	0.434	7.2	0.217	16.1	0.582
41	5-FU/LV	105	15	0.055	5.5	0.0002	12.9	0.16
	5-FU/LV + BV	104	26		9.2		16.6	
43	FOLFOX	289	9	< 0.001	4.8	< 0.001	10.7	0.0018
	FOLFOX + BV	290	22		7.2		12.5	
44	FOLFOX/bFOL/XELOX	147	22-43	NR	6.1-8.7	NR	18.2	NR
	FOLFOX/bFOL/XELOX + BV	213	41-53		8.3-10.3		24.4	
45	FOLFOX/XELOX	701	49	0.99	8.5	< 0.001	-	-
	FOLFOX/XELOX + BV	699	47		11		-	

CRC: Colorectal carcinoma; 5-FU/LV: 5-fluorouracil/leucovorin; IFL: Irinotecan/5-FU/leucovorin; FOLFOX-4: Oxaliplatin/5-FU/leucovorin; BV: Bevacizumab; Pts: Patients enrolled; REF: reference; RR: Response rate; PFS: Progression-free survival; TTP: Time to tumor progression; OS: Overall survival; NR: Not reported.

higher RR [control arm, 17%, (95% CI, 7% to 34%); low-dose arm, 40%, (95% CI, 24% to 58%); high-dose arm, 24%, (95% CI, 12% to 43%)]. Although median survival was 7.7 and 2.3 mo higher in the 5-mg/kg arm and 10-mg/kg arm, respectively, it was not statistically significant. These findings contrast with the effective higher dose administered in other tumors like non-small cell lung cancer (15 mg/kg every three weeks)[33], breast cancer (10 mg/kg every two weeks)[34] and renal cancer (10 mg/kg every two weeks)[35]. Nevertheless, the majority of subsequent CRC studies administered a BV dose of 5 mg/kg. Potential safety concerns observed in this phase Ⅱ study were thrombosis, hypertension, proteinuria, and epistaxis.

In 2004, a large (813 patients) phase Ⅲ, double-blind, randomized trial in patients with untreated metastatic CRC demonstrated that the addition of BV to IFL (irinotecan/5-FU/LV) chemotherapy prolonged OS by 4.7 mo compared with IFL alone (20.3 *vs* 15.6 mo; HR = 0.66, $P < 0.001$)[28]. The one-year survival rate was 74.3% in the group given IFL plus BV and 63.4% in the group given IFL plus placebo ($P < 0.001$). All secondary efficacy end points were also improved with the addition of BV to the chemotherapeutic regimen: PFS increased from 6.2 to

Table 3 Trials for Vatalanib with chemotherapy in metastatic CRC

REF	Regimen	Pts	RR (%)	*P*	PFS or TTP (mo)	*P*	OS (mo)	*P*
58	FOLFOX-4	583	46		7.6			
	FOLFOX-4 + Vatalanib	585	42	NS	7.7	0.118	NR	-
59-60	FOLFOX-4	429	18	NR	4.1	0.026	11.8	0.511
	FOLFOX-4 + Vatalanib	426	19		5.5		12.1	

CRC: Colorectal carcinoma; REF: Reference; Pts: Patients enrolled; FOLFOX-4: Oxaliplatin/5-FU/leucovorin; RR: Response rate; PFS: Progression-free survival; TTP: Time to tumor progression; OS: Overall survival; NS: Statistically nonsignificant; NR: Not reported.

10.6 mo (hazard ratio HR = 0.54; $P < 0.001$), RR increased from 34.8% to 44.8% ($P = 0.004$), and median duration of the response increased from 7.1 to 10.4 mo (HR = 0.62; $P = 0.001$). Grade 3 hypertension was more common during treatment with IFL plus BV than with IFL plus placebo (11.0 percent *vs* 2.3 percent, $P < 0.01$) but it was easily managed with medical treatment. Although the overall incidence of grade 3 or 4 adverse events was higher among patients receiving the combined treatment, the study did not identify hemorrhage, thromboembolism, and proteinuria as possible BV-associated adverse events. Uncommon but serious side-effects of BV included the appearance of gastrointestinal perforations (1.5%), in some instances with fatal outcome[28].

Toxicity derived from antiangiogenic therapy is a main concern in the management of CRC. BV is associated with a two-fold increase in the risk of arterial thromboembolic events, from 2.5% to 5% ($P < 0.01$)[36]. These events consist primarily of acute coronary syndrome, transient ischemic attack and stroke. Patients at risk for these events are those with a prior history of arterial thromboembolism and age older than 65 years. Moreover, BV administration can result in the development of wound dehiscence. However, the risk of wound healing is not increased if the administration of BV with or without chemotherapy is delayed until 28-60 d after primary care surgery[37].

Although the addition of BV to 5-FU-based combination chemotherapy resulted in statistically significant and clinically meaningful improvement in RR, PFS and OS among patients with metastatic CRC, previous studies have suggested that the benefit observed with irinotecan-based schedules might be limited to patients with a performance status (PS) of 0[38]; and certain subgroups, including those with advanced age, impaired PS, low serum albumin, and prior pelvic radiotherapy, may experience significant toxicities when adding irinotecan to 5-5-FU/LV regimens[39]. In this particular population, the combination of BV and 5-FU/LV would remain a potentially useful therapeutic alternative. Two studies led by Kabbinavar *et al*[40,41] addressed this question enrolling patients who were not candidates for irinotecan because of advanced age or poor PS. The results suggested that 5-FU/LV (Roswell Park Schedule[42]) plus BV seems as effective as IFL and might have a better safety profile. Based on all of the previous data, BV became the first anti-VEGF agent to be approved by the FDA for cancer patients.

On June 2006, the FDA granted approval for a labelling extension for BV in combination with intravenous 5-FU-based chemotherapy for the second-line treatment of metastatic CRC. This decision was based on the preliminary results of the E3200 phase Ⅲ trial of the Eastern Cooperative Oncology Group (ECOG). The aim of this randomized, three-arm, multicenter study was to determine the efficacy of infusional 5-FU/LV/oxaliplatin (FOLFOX) with or without BV (10 mg/kg every two weeks) in 829 patients with irinotecan-refractory advanced CRC not previously treated with BV[43]. The median age was 61 years, 49% had an ECOG performance status of 0, and 80% received prior adjuvant chemotherapy. The combination therapy showed an improvement in the OS by 2.1 mo (12.5 *vs* 10.7 mo; $P = 0.0024$) without a significant difference in the toxicity profile. The BV-alone arm was closed at the interim analysis due to a low RR and an apparent lack of activity in this setting. Final analyses of this trial are forthcoming.

Whether the combination of BV with oxaliplatin/5-FU/LV-based chemotherapy regimens will be the best option for first-line therapy for CRC is under investigation in the TREE study[44] and NO16966[45]. The TREE study was previously designed to assess the safety, tolerability and efficacy of each of three oxaliplatin plus fluoropyrimidine regimens without (TREE1 cohort) or with (TREE2 cohort) BV. In the TREE-2 cohort, BV was added to each regimen. With a follow-up of 27 mo, median OS with infusional 5-FU/LV and oxaliplatin (mFOLFOX-6) plus BV was 26.0 mo, 20.7 mo with bolus 5-FU/LV and oxaliplatin (bFOL) plus BV, and 27.0 mo with capecitabine and oxaliplatin (CapeOX) plus BV. Median OS with oxaliplatin-containing regimens without BV in sequential historical cohorts (TREE-1 study), reached 18.2 mo[44]. However, the first large, randomized, multicenter phase Ⅲ trial to evaluate the efficacy of BV in combination with the standard chemotherapy regimen FOLFOX and the XELOX regimen in the first-line treatment of metastatic CRC is the NO16966[45]. Interestingly, in the general treated population, the addition of BV to FOLFOX did not significantly improve PFS (HR = 0.89, $P = 0.1871$). However, 50% of patients discontinued treatment for reasons unrelated to progression of disease. Further analyses focusing on the on-treatment subgroup population revealed that median PFS for XELOX-BV and FOLFOX-BV was 10.4 mo compared to 8.1 mo for XELOX-Placebo and FOLFOX-Placebo (HR = 0.63, $P < 0.0001$). These results demonstrated that the addition of BV to oxaliplatin-based chemotherapy regimens significantly improves PFS. In addition, continuation of BV until disease progression could be necessary to

optimize the contribution of BV to PFS[45].

The activity shown by BV in the metastatic setting justified the evaluation of this antibody in the adjuvant scenario. In the first trial, the National Surgical Adjuvant Breast and Bowel Project C-08 phase Ⅲ trial[46], 2632 patients with stage Ⅱ or Ⅲ colorectal cancer have been randomized to receive mFOLFOX-6 for 12 cycles with or without BV. Patients assigned to BV plus chemotherapy also received an additional 6 mo of BV alone. This trial has already completed accrual. In a second trial recently finished, the AVANT phase Ⅲ study[47], patients with stage Ⅱ or Ⅲ colorectal cancer were randomized to three combination chemotherapy regimens (FOLFOX-4 *vs* FOLFOX-4 plus BV *vs* capecitabine/oxaliplatin plus BV). In addition, a phase Ⅱ clinical trial, the Eastern Cooperative Oncology Group (ECOG) E5202[48], is evaluating the addition of BV in combination with FOLFOX on patients with stage Ⅱ colon cancer at high-risk for recurrence. In conclusion, at this point in time, no evidence supports the actual use of BV in the adjuvant setting in order to prolong survival. The results of these important clinical trials are eagerly awaited.

VATALINIB IN CRC

A second antiangiogenic approach is to target both cancer cells and endothelial cells with small molecules. Similar to BV, VEGFR multitargeted TK inhibitors have been evaluated in combination with chemotherapy in phase Ⅲ trials. The first agent, semaxinib (SU5416, Pharmacia, San Francisco, California) which targets VEGFR-1, VEGFR-2 VEGFR-3, and PDGFR-β did not show any survival benefit when added intravenously to standard chemotherapy in metastatic CRC. In addition worse toxicity in the semaxinib arm was observed[49]. Finally, in a phase I trial that evaluated the combination of semaxinib with cisplatin/gemcitabine in solid tumors, an unexpected high incidence of thromboembolic events was observed which discouraged overall further investigation of this agent[50].

Another novel synthetic agent, with orally bioavailability, vatalanib (PTK787/ZK222584, Novartis, Basel, Switzerland) belongs to the chemical class of aminophthalazines[51]. It is a potent inhibitor of all known VEGFR tyrosine kinases (TK) with greater potency against VEGFR-1 and VEGFR-2[52,53] (Figure 1). It also inhibits other kinases, such as platelet-derived growth factor receptor beta (PDGFR-β) and c-Kit tyrosine kinase. In preclinical studies, vatalanib has shown antitumor activity in subcutaneously implanted human tumor xenografts in nude mice[53]. Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) and pharmacokinetic (PK) data indicated that vatalanib ⩾ 1000 mg total daily dose is the biologically active dose[54] with a terminal half-life of about 6 h. In view of the short half-life of the drug, a phase I study with vatalanib given twice daily was conducted to exploit the theoretical advantage of maintaining constant drug levels[55]. PK data from this study showed that at equivalent daily doses, drug exposure is comparable with the previous once-daily-dosing schedule[54]; however, the trough levels are significantly higher with the bid dosing. Whether this will translate into improved efficacy is unknown at this time.

Vatalanib has been evaluated in two phase Ⅰ / Ⅱ studies as a single daily-dose in combination with FOLFOX or FOLFIRI, as first-line treatment for patients with metastatic CRC[56,57]. In both studies, vatalanib was safe and well tolerated at doses of 1250 mg/d. Ataxia, expressive dysphasia and dizziness were seen at higher doses when administered in combination with FOLFOX and these were considered dose-limiting toxicities. The combination of vatalanib with chemotherapy significantly affected the PK parameters of SN38, the active metabolite of irinotecan. Indeed, the concentration-time curve (AUC) of SN38 was decreased when vatalanib was added to the FOLFIRI regimen. The relevance of this finding needs further investigation.

Two phase Ⅲ studies have evaluated the administration of vatalanib (single daily-dose of 1250 mg/d) in combination with chemotherapy in CRC (Table 3). A first randomized phase Ⅲ trial (CONFIRM-1) compared the efficacy of vatalanib in combination with FOLFOX versus FOLFOX alone in 1168 patients for first-line treatment of metastatic CRC[58]. The results of the primary endpoint of this trial, PFS, showed a modest benefit of adding vatalanib to FOLFOX without achieving statistical significance (HR = 0.88; P = 0.118). OS has not been reported. The adverse events attributable to vatalanib (hypertension, deep-vein thrombosis, diarrhea and dizziness) were generally reversible and similar to other VEGF pathway inhibitors. No increase in bleeding or bowel perforation compared to placebo was observed. The second phase Ⅲ trial (CONFIRM-2) evaluated the efficacy of vatalanib in combination with FOLFOX *versus* FOLFOX alone in 855 patients with irinotecan-refractory advanced CRC[59,60]. PFS was 1.4 mo significantly longer in the vatalanib arm (5.5 mo *vs* 4.1 mo, HR = 0.83; P = 0.026). No improvement in OS was demonstrated. In the CONFIRM-2 trial, the most frequent grade 3/4 events associated with vatalanib were again hypertension (21% *vs* 5%), diarrhea (16% *vs* 8%), fatigue (14.5% *vs* 6.9%), nausea (11% *vs* 5%), vomiting (9% *vs* 5%) and dizziness (9% *vs* 1%). Two hypotheses have been tried to explain why survival was not affected when adding vatalanib in first and second-line therapy. The first one deals with the short half-life of vatalanib. The once-daily administration of the drug might not be the optimal schedule to maintain constant blood levels of vatalanib, although another study refutes this hypothesis[54]. A second one would be the "off-target" effects, such as targeting PDGFR-β. The inhibition of PDGFR-β could interfere with vascular normalization by blocking perivascular cell recruitment and thus impeding the delivery of chemotherapeutics to chemoresponsive tumors[61].

Major *et al*[62] reported a metanalysis by pooling preplanned strata in CONFIRM-1 (C1) and CONFIRM-2 (C2) trials and showed that patients with high LDH (> 1.5 X ULN) experienced the greatest improvement in PFS for C1 (HR = 0.67; P = 0.01) and for C2 (HR = 0.63; P < 0.001). This finding brings forward an eventual role of LDH in angiogenesis-dependent tumor growth and progression in CRC. Previously, the expression of LDH-5, a LDH isoform, has been linked with distant metastases in

CRC and with the expression of hypoxia inducible factor (HIF)[63]. Furthermore, evidence of a biologic link between tumor LDH, hypoxia and activated VEGF pathway has been described in CRC[64]. LDH, being regulated by the same pathway as VEGF, is expected to reflect a subset of tumors with a high likelihood to bear an activated VEGF signalling pathway. Nevertheless, whether LDH can be used as a surrogate marker for screening patients for TK inhibitor therapy remains an open question. Thus, validation of biomarkers of efficacy of anti-VEGF therapy with the aim of identifying responsive patients and predict the optimal biological dose are imperative.

TARGETED THERAPY COMBINATIONS

Growth factors and their receptors play a pivotal role in the regulation of cancer progression and neovascularization[65], stimulating downstream signaling cascades involved in cell proliferation, survival and antiapoptosis. The expression or activation of epidermal growth factor receptor (EGFR) and ErbB2 are altered in many epithelial tumors, and clinical studies indicate that they have an important role in tumor progression[66]. Inhibiting signaling pathways through EGFR and ErbB2 has become a cornerstone in the treatment of a subgroup of patients with non-small cell lung cancer and breast cancer, respectively. In CRC, cetuximab (IMC C225, Erbitux, ImClone, New York, NY), a monoclonal antibody targeting EGFR[67], has been shown to induce apoptosis of CRC cells[68], and cetuximab in combination with irinotecan (in irinotecan-refractory and EGFR expressing metastatic CRC) was found to reverse resistance to irinotecan, producing a 22.9% RR (BOND-1 Trial)[69,70]. These findings have led to the approval of cetuximab for irinotecan-refractory advanced CRC in the Unites States and, more recently, in Europe.

As it is known, the expression of proangiogenic molecules by tumor cells can be stimulated by EGFR receptor signaling[71]. Furthermore, several studies have shown that EGFR inhibitors reduce VEGF and microvessel density in tumors that regress upon EGFR blockade[72,73]. These results provide a strong rationale for combinations of anti-EGFR agents with angiogenesis inhibitors in CRC.

The safety and efficacy of concurrent administration of BV and cetuximab has been evaluated in a randomized phase Ⅱ trial in patients with irinotecan-refractory metastatic CRC (BOND-2 trial)[74]. Seventy-five patients were assigned to receive either irinotecan/cetuximab/BV (5 mg/kg every other week) or cetuximab/BV. This study presents a similar design to BOND-1 trial with BV included in both arms. The combination of cetuximab/BV, alone or with irinotecan, is tolerable, and RR and median TTP seen with the addition of BV to either arm appear favorable compared to historical controls of the BOND-1 trial. The results of the BOND-2 trial validate the design of the planned intergroup trial CALGB/SWOG 80405[75], which plans to randomize 2289 patients to receive standard chemotherapy with the addition of cetuximab, BV, or both monoclonal antibodies in first-line metastatic CRC. The primary end-point of this trial will be to detect differences in overall median survival.

SMALL-MOLECULE TK INHIBITORS IN CRC

Finally, novel VEGFR and/or PDGFR TK inhibitors with different ranges of nanomolar potencies, selectivities, and pharmacokinetic properties are entering phase Ⅰ/Ⅱ trials for the treatment of cancer[76-78]. In addition, there are now available a series of TK inhibitors that block both the EGFR and the downstream signalling molecules on the one hand and the VEGF receptor TK on the other (Table 1). Zactima (ZD6474, AstraZeneca Pharmaceuticals, Cheshire, UK) is an orally bioavailable, anilinoquinazoline derivative, multitargeted tyrosine kinase inhibitor that targets VEGFR-2, EGFR, and RET tyrosine kinases, and is currently in phase Ⅰ/Ⅱ evaluation for the treatment of cancer[79,80]. Another broad spectrum multitargeted agent, AEE788 (Novartis, Basel, Switzerland), is an oral small-molecule inhibitor of both EGFR and VEGFR tyrosine kinases[81,82]. In preclinical studies, this agent has shown growth and metastases inhibition of human colon carcinoma in an orthotopic nude mouse model[83]. Sorafenib (BAY 43-9006; Nexavar®, Bayer Aktiengesellschaft, Leverkusen-Bayerwerk, Germany, and Onyx Pharmaceuticals Inc., Emeryville, CA) targets VEGFR2 and VEGFR3, PDGFR-β, c-Kit and FLT3 (fms-related tyrosine kinase 3) and the downstream signalling molecule of EGFR known as Raf[84]. This agent efficiently inhibits both tumor-cell proliferation and angiogenesis in preclinical models, and monotherapy treatment has shown efficacy in a phase Ⅲ trial in patients with cytokine-refractory advanced renal carcinoma, which led in 2005 to the approval by the FDA for this indication[85]. In contrast with BV, the monotherapy efficacy demonstrated by Sorafenib could mimic the synergistic effect of the combination of an anti-VEGF antibody and chemotherapy[86]. The activity of Sorafenib and similar agents in the treatment of CRC needs further development. In addition, whether it will be better to target the EGFR and VEGF receptor with two compounds, each targeting one system, or to use these new class of oral duals or broad-spectrum inhibitors, is not known at this time[87].

SUMMARY AND CONCLUDING REMARKS

The increased knowledge of the VEGF signaling network and its implication in the development and progression of CRC, together with the initial positive clinical results observed with anti-VEGF therapies, makes angiogenic targeting an appropriate cancer treatment strategy. Based on the results of the completed phase Ⅲ trials, BV can increase survival when combined with standard chemotherapy in first and second-line therapy of advanced CRC. These findings have led to the approval of BV for the treatment of metastatic CRC. Simultaneously, the activity of BV in combination with 5-FU/LV-based chemotherapy regimens is being evaluated in early disease, a period when angiogenesis might be particularly critical. Results of these trials are eagerly awaited. The initial positive results of anti-VEGF therapy are not accomplished without added toxicity. Side effects of anti-VEGF agents are usually moderate compared with other

therapies, but the etiology is poorly understood. Major safety concerns have been raised by increased morbidity, and a number of treatment-related deaths from bowel perforations and cardiovascular events. Modest elevations in blood pressure occur occasionally and are easily managed with standard antihypertensive medications.

Since multiple growth-controlling pathways may be altered in cancer cells, combination antibody strategies are being explored in advanced CRC. BV is being assessed in combination with cetuximab in irinotecan-refractory metastatic CRC, based on the positive results of anti-EGFR therapies in this context. Preliminary data for this combination shows remarkable results without substantial differences about toxicity. New clinical trials with both targeted strategies in first-line metastatic CRC are recruiting patients. Combination of BV with novel VEGFR and broad-spectrum TK inhibitors also needs to be assessed in the treatment of CRC. One of these VEGFR TK inhibitors, vatalanib, combined with standard chemotherapy has been shown not to improve survival in first and second-line treatment of advanced CRC in both phase Ⅲ trials. New broad-spectrum TK inhibitors, such as Sorafenib, oppositely to the VEGF antibody, have shown promising monotherapy activity in other tumors. The basis of these findings is being extensively evaluated, and the identification of biomarkers to predict therapeutic response and optimal doses of anti-VEGF therapy is urgently needed in order to identify patients who will benefit from antiangiogenic therapy.

Angiogenesis research moves in two directions. In one hand, ongoing and new, well-designed trials will define the optimal clinical application of the actual antiangiogenic agents, and, on the other, intensive efforts in basic research will identify new agents with different antiangiogenic approaches for the treatment of CRC.

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