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Targeting Angiogenesis in Esophagogastric Adenocarcinoma

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LEARNING OBJECTIVES

After completing this course, the reader will be able to:

1. Describe the receptors and ligands with identified roles in tumor angiogenesis and the mechanism of action of established and investigational antiangiogenic agents.
2. Describe aspects of antiangiogenic agents that are incompletely understood and need further investigation to define their role in esophagogastric cancer.

ABSTRACT

The possibility of targeting tumor angiogenesis was postulated almost 40 years ago. The vascular endothelial growth factor (VEGF) family and its receptors have since been characterized and extensively studied. VEGF overexpression is a common finding in solid tumors, including esophagogastric cancer, and frequently correlates with poor prognosis. Monoclonal antibodies, soluble receptors, and small-molecule tyrosine kinase inhibitors have been

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developed to inhibit tumor angiogenesis, and antiangiogenic therapy is now a component of standard treatment for advanced renal cell, hepatocellular, colorectal, breast, and non-small cell lung carcinomas. The small-molecule tyrosine kinase inhibitors sunitinib and sorafenib have been evaluated in phase II studies in esophagogastric cancer but appear to have only modest activity. Similarly, despite promising efficacy signals from phase II studies, the addition of the anti-VEGF-A monoclonal antibody bevacizumab to cisplatin plus capecitabine failed to result in a longer overall survival duration than with the chemotherapy doublet plus placebo. The response rate and progression-free survival interval were significantly greater with bevacizumab, confirming some efficacy in advanced gastric cancer, but with inadequate benefit to justify the high cost of treatment. Evaluation of bevacizumab in the neoadjuvant and perioperative settings continues, hypothesizing that a higher response rate will translate into longer survival in patients with operable disease. Despite extensive research, the discovery of a reliable predictive biomarker for antiangiogenic therapy continues to elude the scientific and oncology communities, and mechanisms of primary and acquired resistance are incompletely understood. We are therefore currently unable to personalize antiangiogenic therapy for established indications, or use molecular selection for clinical trials evaluating novel indications.
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INTRODUCTION

Gastric and esophageal cancers are the fourth and eighth most common cancers worldwide, with a combined annual incidence of almost 1.5 million cases and resulting in >1 million deaths per year [1]. For patients with operable disease, multimodality therapy is an internationally accepted standard, because surgery alone results in relatively poor long-term survival. Perioperative chemotherapy [2], adjuvant chemotherapy [3], and chemoradiation [4] produce longer overall survival (OS) times for gastric cancer patients. Similarly, perioperative chemotherapy [2], neoadjuvant chemotherapy [5], and chemoradiation [6] lead to longer OS times for patients with operable esophageal adenocarcinomas.

The majority of patients presenting with esophagogastric carcinoma have advanced disease, with a median survival time of ~3 months with supportive care alone [7]. With combination chemotherapy, median survival times of 9 and 14 months have been reported for patients with metastatic and locally advanced inoperable disease, respectively [8]. There is no international consensus regarding the optimal first-line chemotherapy regimen; however, treatment with a platinum and fluoropyrimidine doublet [9] or a triplet regimen with the addition of epirubicin [10] or docetaxel [11] is most frequently used. Following successful results in other solid tumors, targeted agents are now being evaluated in esophagogastric cancer. The recent positive results from the randomized phase III ToGA (Trastuzumab for Gastric Cancer) study have changed the treatment paradigm for patients with human epidermal growth factor receptor 2–positive disease, for whom treatment with a platinum and fluoropyrimidine doublet plus trastuzumab is now the standard of care [12].

The second targeted agent to undergo phase III evaluation in advanced esophagogastric cancer was an antiangiogenic agent, bevacizumab. Angiogenesis is an essential event for small, established tumors to grow beyond a critical size of a few millimeters. It is thought that without the necessary microenvironment for neovascularization, tumor growth is arrested. This proposed dependency on angiogenesis, in addition to the lack of angiogenesis in normal tissues under physiological conditions other than embryogenesis, the female menstrual cycle, wound healing, and muscle growth, made angiogenesis a logical therapeutic target, with minimal toxicity to normal tissues expected [13].

Bevacizumab, a monoclonal antibody directed against vascular endothelial growth factor (VEGF)-A, was the first antiangiogenic drug to be clinically evaluated and was licensed for the first-line treatment of patients with metastatic colorectal cancer (mCRC) following the report of an almost 5-month survival benefit when added to irinotecan and 5-fluorouracil (5-FU) [14]. Small-molecule inhibitors of VEGF receptor (VEGFR) tyrosine kinase activity, sunitinib and sorafenib, have since been established as standard first- and second-line therapies, respectively, for patients with metastatic renal cell carcinoma (RCC) [15, 16]. Sorafenib also leads to longer survival in patients with metastatic hepatocellular carcinoma (HCC) and is a standard first-line therapy [17]. Both drugs exert at least part of their therapeutic activity via inhibition of VEGFRs.

This review focuses on the rationale for targeting angiogenesis in oncology and the current and possible future applications of antiangiogenic agents in esophagogastric adenocarcinoma.

THE VEGF FAMILY

VEGF-A

VEGF-A is secreted by several human and rodent tumor cell lines [18] and was shown to stimulate endothelial cell

growth and angiogenesis [19]. Serum VEGF-A levels in patients with cancer are often higher than normal physiological levels [20]. There are at least 12 isoforms of VEGF-A, although the soluble VEGF-A_{121} and VEGF-A_{165} isoforms have been most studied [21]. VEGF-A mediates its effects by binding to two endothelial cell surface tyrosine kinase receptors, VEGFR-1 and VEGFR-2, but activation of VEGFR-2 is considered to be more critical to angiogenesis [22]. Proof of concept for the therapeutic activity of VEGF inhibition was reported in 1993, when a monoclonal antibody directed at VEGF-A was reported to inhibit angiogenesis and tumor growth in human tumor xenografts. That group successfully humanized the antibody, which became known as bevacizumab [23].

Regulation of VEGF-A Expression

Transcription of the gene encoding VEGF-A is mediated by hypoxia inducible factor (HIF)-1, a heterodimeric protein composed of α and β subunits. Under normoxic conditions, prolyl hydroxylase domain proteins hydroxylate the oxygen-dependent degradation domain of HIF-1α, precipitating interaction with the von Hippel Lindau (VHL) protein and subsequent degradation of HIF-1α. A second regulator of HIF-1α, known as factor inhibiting HIF-1, also prevents activation of the HIF pathway in well-oxygenated cells, via hydroxylation of the transcriptional activation domain. However, under hypoxic conditions, neither enzyme is able to hydroxylate its target on HIF-1α, allowing transcription of hypoxic response genes, including *VEGF-A* [24]. Targeted inhibition of HIF-1α inhibits tumor growth and angiogenesis in animal models, providing a rationale for therapeutic targeting of HIF-1α in oncology [25].

The efficacy of antiangiogenic agents in RCC patients is likely to relate to the frequent inactivation of the *VHL* gene, accumulation of HIF-1α, and subsequent overexpression of VEGF-A and other proangiogenic factors [26].

Placental Growth Factor, VEGF-B, VEGF-C, VEGF-D, and VEGF-E

Placental growth factor (PlGF) mediates the angiogenic response to VEGF by activating VEGFR-1 [27] and regulating crosstalk between VEGFR-1 and VEGFR-2 [28]. Direct anti-PlGF targeting is of particular interest because of the lack of effect on normal vessels coupled with the activity of an anti-PlGF antibody, 5D11D4, reported in VEGF-resistant tumors [29]. However, these data were recently challenged by a report of impaired wound healing but no inhibition of angiogenesis or growth in tumors by four novel anti-PlGF antibodies [30]. Further preclinical studies of 5D11D4 have confirmed the antitumor effect of this antibody in HCC [31], but the reason for the inconsistent efficacy in preclinical models remains unclear.

VEGF-C is normally expressed in multiple human tissues and preferentially binds to VEGFR-3, although it also binds to and activates VEGFR-2, albeit with lower affinity [32]. VEGF-C expression in animal studies is associated with the frequent development of lymph node metastases [33]. Similarly, detection of VEGF-C in a study of 139 resected gastric cancers with submucosal invasion was significantly associated with the presence of lymph node metastases on multivariate analysis (odds ratio, 4.18; 95% confidence interval [CI], 1.38–12.7; $p = .0116$) [34].

VEGF-B activates VEGFR-1 but has little angiogenic activity outside the myocardium, where loss of VEGF-B impairs angiogenesis in the ischemic heart [35]. VEGF-D activates VEGFR-2 and VEGFR-3 and stimulates the growth of endothelial cells in vitro, but is approximately five times less potent than VEGF-A and therefore may be a less important therapeutic target [36] VEGF-E appears to bind only to VEGFR-2 and has similar proangiogenic activity to that of VEGF-A [37], but the gene encoding VEGF-E is not present in the human genome and it is therefore unlikely to have a role in cancer treatment.

VEGF Receptors

VEGFR-1, VEGFR-2, and VEGFR-3

VEGFR-1 through VEGFR-3 are receptor tyrosine kinases that are expressed by vascular and lymphatic endothelial cells, and their expression has also been identified on several normal embryological and adult tissues as well as tumor cells [22]. Figure 1 depicts VEGFRs and downstream signaling pathways.

VEGFR-2 is considered to be the principal receptor by which VEGF-A induces angiogenesis. The downstream effects of VEGFR-2 activation are mediated by several signaling pathways, including the phospholipase C (PLC)-γ, protein kinase C (PKC), extracellular signal–related kinase (ERK), phospatidylinositol 3-kinase (PI3K), and endothelial nitric oxide synthase (eNOS) pathways [22]. Inhibition of VEGFR-2 was shown to suppress angiogenesis and tumor growth in numerous preclinical models, validating it as a potential target [38, 39].

Despite high-affinity binding to VEGF-A, the level of VEGFR-1 kinase activity is low. Downstream signaling pathways are ill defined, but VEGF induces phosphorylation of PLC-γ, PI3K, PKC, and ERK/mitogen-activated protein kinase (MAPK) [22]. It is thought that VEGFR-1 may act as a decoy receptor, thereby regulating the VEGF-A available to bind VEGFR-2 [22], or act to refine

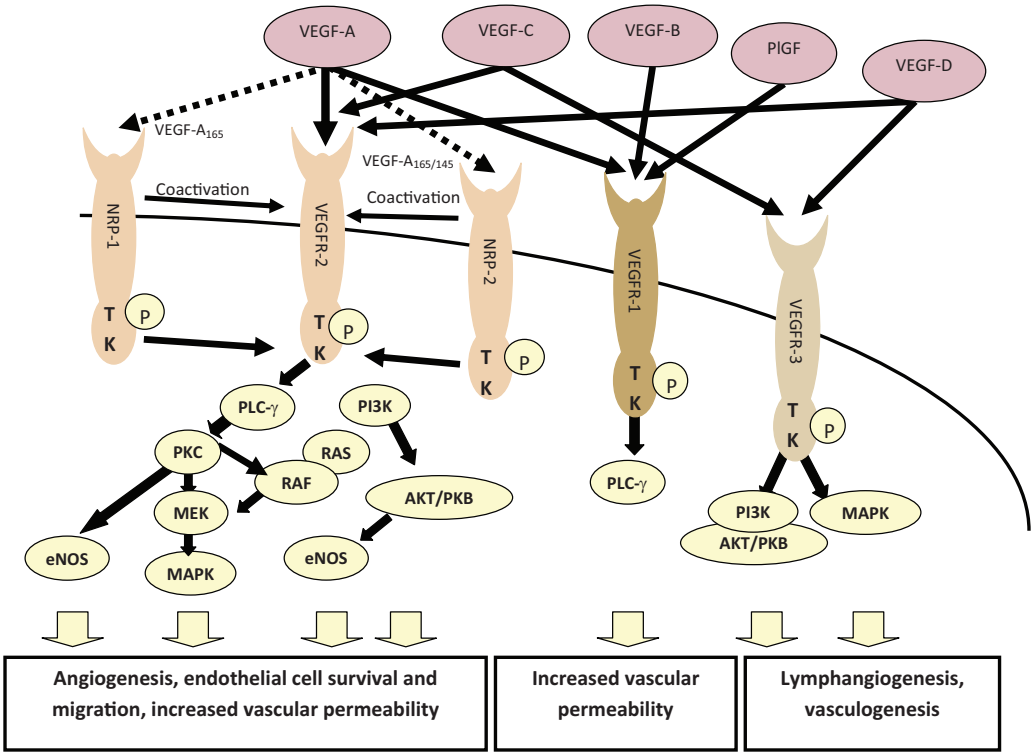

Figure 1. The three VEGF receptors, two coreceptors, and downstream signaling pathways. VEGF-A binds to VEGFR-1 and VEGFR-2, with additional isoform-specific binding to the NRP receptors, which coactivate VEGFR-2. VEGF-B and PlGF bind to VEGFR-1, and VEGF-C and VEGF-D both bind to VEGFR-3 and VEGFR-2. Activation of these receptors stimulates a signaling cascade resulting in angiogenesis, increased vascular permeability, and lymphangiogenesis.

Abbreviations: eNOS, endothelial nitric oxide synthase; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal–related kinase kinase; NRP, neuropilin; PI3K, phospatidylinositol-3-kinase; PKB, protein kinase B; PKC, protein kinase C; PLCγ, phospholipase Cγ; PlGF, placental growth factor; TK, tyrosine kinase; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

VEGF signaling by heterodimerization with VEGFR-2 [28].

VEGFR-3 is widely expressed in benign and malignant vascular tumors, but not in solid tumors, including undifferentiated carcinomas, in which only the capillaries at the site of neovascularization stain for VEGFR-3 [40]. Downstream signaling via PKC-dependent MAPK activation has been reported in lymphatic endothelial cells [41] and in the Ras–MAPK pathway in human hematopoietic cells [42], but these pathways have not been fully defined. Blockade of VEGFR-3 using a soluble fusion protein, VEGFR-3 immunoglobulin, in a human lung cancer cell line xenograft suppressed tumor lymphangiogenesis and lymph node metastasis but not visceral metastasis [43], suggesting that dual targeting of VEGFR-3 and VEGFR-2 may be valuable.

Several small-molecule inhibitors of VEGFR tyrosine kinase activity have also been developed, including sunitinib, a multi–tyrosine kinase inhibitor (TKI) that potently inhibits VEGFR-1, VEGFR-2, VEGFR-3, platelet-derived growth factor receptors (PDGFRs), and the Kit receptor. Several other TKIs have been evaluated, with those reaching clinical testing including sorafenib, pazopanib, cediranib (AZD2171), and axitinib (AG-013736) [44].

Neuropilin 1 and Neuropilin 2

Neuropilin (NRP)-1 is a molecule that may play multiple roles in angiogenesis. It is perhaps best known as an isoform-specific coreceptor for VEGF-A_{165} and may promote signaling through VEGFR-2 when the two receptors are coexpressed [45]. However, NRP-1 also mediates signaling of the semaphorins, which may be involved in inhibition of vessel formation [46], and can act as a cell adhesion receptor [47]. NRP-1 can also be expressed in tumor cells. Overexpression of NRP-1 in pancreatic cancer cell lines induces MAPK signaling and is associated with resistance to gemcitabine and 5-FU chemotherapy [48]. Inhibition of NRP-1 suppresses neovascularization in animal models [49], demonstrating the potential of the receptor as a therapeutic target. A small-molecule inhibitor of VEGF-A binding to NRP-1, EG00229, has in vitro activity in lung cancer cell

lines, and an apparent synergistic effect with paclitaxel and 5-FU has been reported [50].

NRP-2 is also isoform specific for VEGF-A, binding only the $VEGF_{165}$ and $VEGF_{145}$ isoforms, but it also binds PlGF [51]. VEGF-A and VEGF-C can induce interaction of NRP-2 with VEGFR-2, enhancing VEGFR-2 signaling and consequent endothelial cell survival [52]. VEGF-C also weakly induces NRP-2 interaction with VEGFR-3 [52]. Inhibition of NRP-2 in colorectal cancer cell lines leads to impaired tumor growth both in vitro and in vivo [53]. NRP-2 is upregulated in gastric cancer endothelial cells, enhancing the proproliferation and migration effects of VEGF [54].

Clinical Data in Esophagogastric Cancer

Monoclonal Antibodies: Bevacizumab

Bevacizumab first underwent phase III evaluation in mCRC patients, with a significant OS benefit reported [14], precipitating licensing worldwide for this indication. The optimal duration of bevacizumab treatment has not yet been established in this setting, with data from an observational study suggesting that treatment beyond disease progression is associated with longer survival [55], but there are no confirmatory data from a randomized study. Combination with chemotherapy appears necessary for mCRC patients, with minimal monotherapy activity reported in a second-line study [56]. In the curative-intent setting, the addition of bevacizumab to adjuvant 5-FU, leucovorin, and oxaliplatin (FOLFOX) did not result in a longer disease-free survival interval in patients with resected stage II–III colon cancer [57]. Moreover, in the AVANT (Avastin as Chemotherapy for Adjuvant Colon Carcinoma) study of bevacizumab added to adjuvant FOLFOX or capecitabine plus oxaliplatin, both the disease-free and preliminary OS results favored the control arm. Although greater use of bevacizumab after disease progression in the control arm may account, in part, for the longer survival time in that group, it cannot explain the apparent detrimental effect on disease-free survival [58]. This lack of benefit in early disease, on the background of proven efficacy in advanced disease, was previously reported with both the chemotherapeutic agent irinotecan [59, 60] and the targeted agent cetuximab [61]. This suggests significant differences between early and advanced stage disease in terms of drug sensitivity and molecular alterations. Correlative translational work from these adjuvant studies may yet define subgroups of patients who benefit from such agents. Bevacizumab is additionally licensed in combination with interferon for the treatment of patients with advanced RCC, and with chemotherapy for the treatment of patients with advanced breast and non-small cell lung cancers.

In esophagogastric cancer, response rates (RRs) of 65%–68% were reported in three phase II studies of bevacizumab with combination chemotherapy [62–64], with encouraging median progression-free survival (PFS) and OS times reported in combination with irinotecan and cisplatin [64]. However, a further phase II study reported more modest efficacy in combination with oxaliplatin and docetaxel [65], comparable with the results with chemotherapy triplet regimens in phase III studies [10, 11]. Unfortunately, these results reflect those reported in the international, randomized phase III AVAGAST (Avastin for Advanced Gastric Cancer) study, in which 774 patients with advanced gastric or esophagogastric junction (EGJ) adenocarcinoma were randomized to a cisplatin–fluoropyrimidine doublet with bevacizumab or placebo in just 14 months. Although bevacizumab showed efficacy in this disease in terms of a higher RR and longer median PFS interval, the study failed to meet the primary endpoint of a statistically significant longer OS duration. Possible regional variation in efficacy was reported in a subgroup analysis, with an apparent benefit noted in patients treated in pan-America but no benefit in those treated in Asia [66]. One possible explanation for the apparent geographical variation is the wide variation in the use of second-line chemotherapy, whereby the highest rates were reported in Asian patients (66%) and the lowest rates were reported in pan-American patients (21%). In view of the hypothesis-generating data from mCRC [55], it may be valuable to evaluate bevacizumab beyond disease progression in combination with second-line chemotherapy in a randomized study in advanced gastric cancer.

Bevacizumab was also evaluated in a small phase II study in the second-line treatment of esophagogastric cancer. An encouraging RR of 27% was reported in combination with weekly docetaxel in the 20 evaluable patients, and the final results of the study are awaited [67]. These results are summarized in Table 1.

Initial phase I studies of bevacizumab as monotherapy [68] and in combination with three chemotherapy regimens [69] showed no dose-limiting toxicities, and it was not until phase II and phase III evaluation in mCRC patients that the characteristic toxicities of bevacizumab, including hypertension, proteinuria, arterial and venous thromboembolism, and gastrointestinal perforation, were recognized [14, 70]. In a phase II study combining bevacizumab (15 mg/kg) with irinotecan and cisplatin every 3 weeks in patients with advanced gastric cancer, grade 3–4 venous thromboembolic events were reported in 25.5% of patients, myocardial infarction was reported in one patient, and gastric perforation was re-

Table 1. Phase II/III trials of antiangiogenic drugs in advanced esophagogastric cancer

Trial	Eligible patients	Treatment	*n* of patients	Response rate	Median PFS (95% CI), mos	Median OS (95% CI), mos
First line						
Phase II, Sun et al. (2010) [75]	Locally advanced or metastatic gastric or EGJ adenocarcinoma	Docetaxel (75 mg/m^2) + cisplatin (75 mg/m^2) on day 1 + sorafenib (400 mg) twice daily days 1–21 every 21 days	44	41%	5.8 (5.4–7.4)	13.6 (8.6–16.1)
Phase II, Shah et al. (2006) [64]	Metastatic or unresectable gastric or EGJ adenocarcinoma	Irinotecan (65 mg/m^2), cisplatin (30 mg/m^2) on days 1 and 8 + bevacizumab (15 mg/kg) on day 1 every 21 days	47	65%	8.3 (5.5–9.9)	12.3 (11.3–17.2)
Phase II, Enzinger et al. (2008) [62]	Metastatic esophagogastric cancer	Docetaxel (30 mg/m^2) + cisplatin (25 mg/m^2) + irinotecan (50 mg/m^2) on days 1 and 8 + bevacizumab (10 mg/kg) every 21 days	26	68%	Not reported	Not reported
Phase II, Shah et al. (2011) [63]	Metastatic gastric or EGJ adenocarcinoma	Docetaxel (40 mg/m^2) on day 1, 5-FU (400 mg/m^2 on day 1 + 2,000 mg/m^2 over 48 hrs) + leucovorin (400 mg/m^2) on day 1, cisplatin (40 mg/m^2) on day 3 + bevacizumab (10 mg/kg) on day 1 every 14 days	44	67%	12 (8.8–18.2)	16.8 (12.1–26.1)
Phase II, El-Rayes et al. (2010) [65]	Locally advanced or metastatic gastric or EGJ adenocarcinoma	Oxaliplatin (75 mg/m^2), docetaxel (70 mg/m^2) + bevacizumab (7.5 mg/kg) on day 1 every 21 days	38	42%	6.6 (4.4–10.5)	11.1 (8.2–15.3)
Phase III, AVAGAST, Kang et al. (2010) [66]	Metastatic or inoperable locally advanced gastric or EGJ adenocarcinoma	Cisplatin (80 mg/m^2) on day 1, capecitabine (2,000 mg/m^2 per day) on days 1–14 + placebo on day1 every 21 days versus cisplatin (80 mg/m^2) on day 1, capecitabine (2,000 mg/m^2 per day) on days 1–14 + bevacizumab (7.5 mg/kg) on day 1 every 21 days	387 versus 387	37% versus 46% (p = .032)	5.3 versus 6.7; HR, 0.80; CI, 0.68–0.93 (p = .004)	10.1 versus 12.1; HR, 0.87; CI, 0.73–1.03 (p = .100)
Second line						
Phase II, Bang et al. (2007) [76]	Stage IV advanced gastric cancer	Sunitinib (50 mg/day) on days 1–28 every 42 days	78	2.6%	2.3	6.8
Phase II, Enzinger et al. (2006) [67]	Metastatic esophageal and gastric cancer (1 patient with SCC included)	Docetaxel (35 mg/m^2) on days 1, 8, and 15 + bevacizumab (5 mg/kg) on days 1 and 15, every 28 days	20	27%	Not reported	Not reported

Abbreviations: 5-FU, 5-fluorouracil; AVAGAST, Avastin as Chemotherapy for Adjuvant Colon Carcinoma; CI, confidence interval; EGJ, esophagogastric junction; HR, hazard ratio; SCC, squamous cell carcinoma.

ported in two of 47 patients [64]. These unexpectedly high rates of thromboembolic events were inconsistent with published phase III studies of bevacizumab for other solid tumors and may relate, in part, to better detection of asymptomatic pulmonary emboli on computed tomography CT scans. However, additionally, the underlying disease, the irinotecan-based chemotherapy regimen, and possibly the bevacizumab dose may also have contributed. In support of this, in the AVAGAST study, in which bevacizumab (7.5 mg/kg every 3 weeks) was delivered with a cisplatin–fluoropyrimidine doublet, there was not a higher incidence of venous or arterial

thromboembolic events than in patients treated with chemotherapy plus placebo. The rates of other expected bevacizumab-related toxicities, including hypertension, bleeding, wound-healing events, and gastrointestinal perforations, were higher in the bevacizumab arm, but the rates of serious complications were low. The recognized rare toxicities of fistula or abscess formation and reversible posterior leukoencephalopathy syndrome were each reported in two of 386 patients randomized to receive bevacizumab [66].

The evaluation of bevacizumab in localized esophagogastric cancer is ongoing in phase II and phase III studies, and only safety data are available. Of 14 evaluable patients treated with bevacizumab (7.5 mg/kg every 3 weeks) in combination with weekly irinotecan and cisplatin chemoradiation, 10 underwent surgery, in whom there were no unexpected surgical or wound-healing problems, but anastomotic leaks were reported in two patients (20%) [71]. In contrast, in the phase II/III ST03 study of perioperative epirubicin, cisplatin, and capecitabine with or without bevacizumab (7.5 mg/kg every 3 weeks), preliminary safety data from the first 104 patients randomized showed no difference in the incidence of wound-healing complications or anastomotic leaks and similar rates of thromboembolic events [72]. Current phase III studies evaluating antiangiogenic agents in esophagogastric cancer patients are listed in Table 2.

VEGFR Antibodies: Ramucirumab (IMC1121B)

Ramucirumab is a fully human IgG_1 monoclonal antibody to VEGFR-2. A phase I study of 37 patients with previously treated advanced solid tumors identified a safety profile similar to that of bevacizumab, with serious adverse events including dose-related hypertension, venous thromboembolism, and proteinuria reported. The maximum tolerated weekly dose was 13 mg/kg, although pharmacokinetic studies demonstrated that clearance of the drug was saturated at 8 mg/kg. Partial responses were observed in four patients, including one with previously treated gastric cancer [73]. Phase III evaluation of ramucirumab as monotherapy and in combination with weekly paclitaxel in previously treated advanced gastric cancer patients is under way.

TKIs

Sorafenib is an oral multitargeted TKI that inhibits VEGFR-1, VEGFR-2, and VEGFR-3, PDGFRs, B-Raf, Raf-1, and c-Kit. Sorafenib monotherapy led to a longer OS time in metastatic HCC patients [17] and a longer PFS interval as second-line therapy in metastatic RCC patients [15]. Common toxicities included diarrhea, fatigue, hypertension, hand–foot syndrome, rash, alopecia, anorexia, and nausea. Serious cardiotoxicity, such as myocardial ischemia or infarction, is rare [15]. In gastric cancer, a phase I evaluation of sorafenib plus capecitabine and cisplatin defined diarrhea and neutropenia as dose-limiting toxicities, with an encouraging RR (62.5%), median PFS duration (10 months; 95% CI, 7.4–13.8 months), and median OS duration (14.7 months; 95% CI, 12.0–20.0 months) reported in the 21 patients enrolled [74]. A subsequent phase II study of sorafenib with 3-weekly docetaxel and cisplatin reported possible additive efficacy, with a median OS time of 13.6 months (90% CI, 8.6–16.1 months). However, the median PFS time of 5.8 months (90% CI, 5.4–7.4 months) is less than that reported in a phase III study of chemotherapy alone [10] and could suggest that the longer OS duration reflects the use of second-line chemotherapy [75].

Sunitinib also targets VEGFRs among other intracellular targets and led to a higher RR and longer PFS interval as monotherapy for the first-line treatment of advanced RCC patients [16]. Frequently reported adverse events include those of sorafenib, but, additionally, neutropenia and biochemical abnormalities such as elevated serum lipase are common [16]. A single-arm phase II study of sunitinib monotherapy in 78 patients with previously treated gastric or EGJ adenocarcinoma reported a disappointing radiological RR of 2.6%, median PFS time of 2.3 months (95% CI, 1.6–2.6 months), and median OS time of 6.8 months (95% CI, 4.4–9.7 months), demonstrating modest efficacy in this disease setting [76]. Phase III evaluation compared with supportive care alone is anticipated.

Other VEGFR TKIs—axitinib, vatalinib, cediranib, and pazopanib—have not yet been evaluated in patients with esophagogastric cancer.

Other Potential Antiangiogenic Therapies for Esophagogastric Cancer

Aflibercept (VEGF Trap) binds to and inactivates circulating VEGF-A and PlGF, with higher affinity for VEGF-A than bevacizumab, and has undergone phase I and phase II evaluation in several solid tumors. Of interest, in previously treated mCRC patients, monotherapy activity was apparent in patients who had received prior bevacizumab [77]. The fully human anti–NRP-1 antibody MNRP1685A is currently undergoing phase Ib evaluation in combination with bevacizumab and with weekly paclitaxel [78]. An oral inhibitor of HIF-1α, PX-478, has undergone phase I testing in patients with advanced solid tumors [79]. However, none of these agents have yet been evaluated in esophagogastric cancer.

Table 2. Current phase III trials of antiangiogenic agents in esophagogastric cancer

Trial name	Planned recruitment	Eligibility	Status	Treatment
Operable disease				
ST03	1,100	Stage Ib–IV resectable adenocarcinoma of the stomach, lower esophagus, and EGJ	Open to recruitment in the U.K.	Perioperative ECX ×3 cycles before and after surgery versus perioperative ECX + bevacizumab ×3 cycles before and after surgery, then maintenance bevacizumab ×6 cycles
Advanced disease				
ClinicalTrials.gov identifier, NCT00887822	200	First-line Chinese patients with advanced gastric cancer	Completed recruitment in China	Cisplatin (80 mg/m^2) on day 1, capecitabine (2,000 mg/m^2 per day) on days 1–14 + placebo on day 1 every 21 days versus cisplatin (80 mg/m^2) on day 1, capecitabine (2,000 mg/m^2 per day) on days 1–14 + bevacizumab (7.5 mg/kg) on day 1 every 21 days
IMCL CP12–0715	315	Second-line, previously treated metastatic gastric or EGJ adenocarcinoma	Open to recruitment internationally	Ramucirumab (IMC 1121B) (8 mg/kg) every 14 days + best supportive care until disease progression versus placebo + best supportive care until disease progression
IMCL CP12–0922	663	Second -line, previously treated metastatic gastric or EGJ adenocarcinoma	Open to recruitment internationally	Paclitaxel (80 mg/m^2) on days 1, 8, and 15 + ramucirumab (IMC 1121B) (8 mg/kg) on days 1 and 15 every 28 days until disease progression versus paclitaxel (80 mg/m^2) on days 1, 8, and 15 + placebo on days 1 and 15 every 21 days until disease progression
ClinicalTrials.gov identifier, NCT00970138	114	Third-line, previously treated with two lines of therapy for metastatic gastric cancer	Open to recruitment in China	Apatinib (850 mg) orally daily + placebo orally daily until disease progression versus apatinib (425 mg) twice daily until disease progression versus placebo twice daily orally until disease progression

Abbreviation: ECX, epirubicin, cisplatin and capecitabine; EGJ, esophagogastric junction.

Evidence for Tumor Rebound Effects and Greater Tumor Aggressiveness in Response to VEGF-Targeted Therapies

A study of s.c. Lewis lung carcinoma cell lines in mice treated with an anti-VEGF TKI showed rapid regrowth of the tumor vasculature after withdrawal of the TKI [80]. These data suggest that continuous administration of antiangiogenic therapy may be necessary for maximum efficacy and to avoid rebound growth of tumors. There have been several preclinical reports of a paradoxical increase in local tumor invasion and development of distant metastases apparently induced by antiangiogenic therapy. A study using metastatic breast cancer and melanoma xenografts treated with sunitinib reported the worrying observation that treatment with TKIs resulted in a higher incidence of metastasis and shorter survival time [81]. This effect is not limited to small-molecule TKIs, because in a murine model of pancreatic neuroendocrine tumors, antibody-mediated blockade of VEGFR-2 (DC101) increased the invasiveness of the tumors and there were more involved lymph nodes on histological examination after 1 or 4 weeks of treatment, despite a smaller tumor volume and longer OS time in treated mice than in controls [82]. However, this is in contrast to a study using an anti-VEGF antibody, in which slower regrowth after anti-VEGF antibody monotherapy was reported, and suppression of a rebound growth effect was noted after discontinuation of chemotherapy when the antibody was delivered concurrently [83].

In clinical practice, there are very limited data to support a "rebound phenomenon" after cessation of antiangiogenic monotherapy. A retrospective study of 12 patients with RCC treated with sunitinib or sorafenib (with or without surgery) to complete response showed disease relapse in five patients within 8 months of discontinuation of the drug, all of whom responded to reintroduction of the TKI [84]. A case series of 53 patients with high-grade gliomas reported rapid regrowth in 11 of the 40 patients with disease progression after cessation of bevacizumab, with an apparent sur-

vival advantage in four of the 11 patients retreated with bevacizumab [85]. However, a recent meta-analysis of 4,205 patients treated in five randomized studies assessed time to disease progression or death after cessation of bevacizumab or placebo prior to disease progression and demonstrated no detrimental effect in patients who received bevacizumab [86]. At present, there is no clinical evidence that rebound growth is a consequence of antiangiogenic therapy or of any negative effect of antiangiogenic therapy on survival.

Predictive Markers of Response

Despite extensive preclinical and clinical research, there currently are no validated biomarkers to select patients for antiangiogenic therapy. However, several candidate surrogate markers of response to bevacizumab have been identified from clinical trials.

Tumor VEGF

Tumor VEGF expression was first identified as a marker of poor prognosis in gastric cancer patients when a significant correlation between VEGF expression and the presence of lymphatic and vascular invasion, lymph node and liver metastases, and OS was observed in a study of 129 patients with gastric cancer resection ($p <$ .05 for each comparison) [87]. Tumor expression of VEGF-D and VEGFR-3 were also reported as independent prognostic markers in a study of 91 patients with gastric adenocarcinoma undergoing complete resection. The carcinoma-specific survival rate was significantly shorter in patients with VEGF-D (relative risk, 3.08; 95% CI, 1.22–7.80; $p = .017$) or VEGFR-3 (relative risk, 2.36; 95% CI, 1.174.74; $p = .016$) expression [88]. A small retrospective study also identified tumor VEGF-C expression as a marker of poor prognosis in patients with resected gastric cancer [89]. Prospective validation of these possible prognostic biomarkers is warranted.

Circulating Angiogenic Factors

Several clinical trials have reported contrasting results when evaluating circulating VEGF as a possible predictive or prognostic biomarker [90–94]. These differences may relate to the assays used, disease setting, tumor type, or treatment regimen. However, a recent analysis of phase III studies across three tumor types demonstrated that circulating VEGF is prognostic, with high levels correlating with shorter PFS and OS times irrespective of treatment with bevacizumab, rather than predictive of response to bevacizumab [95].

Soluble VEGFR-1 was evaluated as a possible predictive biomarker in a phase I/II study of 32 patients treated with neoadjuvant bevacizumab plus 5-FU chemoradiation for T3–4 adenocarcinoma of the rectum. A high plasma VEGFR-1 level at baseline was correlated with a higher pT-stage at surgery [94] ($p < .05$), higher Mandard regression score ($p < .01$), and lower risk for serious adverse events ($p < .05$), suggesting that such patients are refractory to both the therapeutic and toxic effects of bevacizumab [96]. However, this result has not been reproduced in other studies and prospective validation of this potential biomarker in a larger study is warranted. Baseline circulating biomarkers evaluated for bevacizumab are summarized in Table 3.

Genetic Polymorphisms

VEGF genotyping was investigated in the phase III E1200 study in advanced breast cancer patients, showing a longer OS duration in patients with the *VEGF-2578-AA* or *VEGF-1154-A* alleles treated with bevacizumab, but not in those treated with chemotherapy alone [97].

A recent study of angiogenesis-related genetic polymorphisms in resected esophageal cancer patients reported no correlation between *VEGF* or *VEGFR-2* polymorphisms and relapse or survival. A predictive effect could not be evaluated because no patient received antiangiogenic agents. Two independent markers of poor prognosis were identified, however. Polymorphisms in the gene encoding a receptor involved in VEGF regulation, proteinase-activated-receptor 1 (PAR-1 −506 any insertion allele), and the epidermal growth factor (EGF +61 A>G (A/A)) were correlated with a higher risk for disease recurrence [98].

CEPs and CECs

Candidate predictive biomarkers include the quantification of circulating endothelial progenitor cells (CEPs) and circulating endothelial cells (CECs). Bone marrow-derived CEPs, or "angioblasts," were first reported to be incorporated into sites of active angiogenesis, where they differentiate into endothelial cells [99]. These CEPs may regulate the angiogenic switch, promoting angiogenesis-mediated progression of micrometastases. Blockade of CEP mobilization blocked angiogenesis and tumor growth [100], inhibited progression of metastatic disease, and prolonged survival in animal models [101]. CEPs have been reported to be mobilized during neoadjuvant chemotherapy [102], an effect not seen using metronomic dosing [103]. Conflicting results exist regarding the association between high baseline levels of CEPs and response to chemotherapy plus bevacizumab [104–106]. CEPs were recently described in patients with advanced gastric cancer undergoing chemotherapy [107]; therefore, prospective evaluation of CEPs as a possible marker of response to bevacizumab in this population may be feasible.

CECs were identified in the plasma of cancer patients,

Table 3. Possible baseline plasma or serum biomarkers for bevacizumab evaluated in clinical trials

Disease setting, study	Treatment regimen	*n* of patients	Biomarker	Effect on clinic outcome
mCRC, Ronzoni et al. (2010) [105]	Bevacizumab (5 mg/kg) every 14 days or 7.5 mg/kg every 21 days + FOLFIRI, FOLFOX, XELOX or FOLFOXIRI	40	Resting CECs; total CECs, CEPs	Responding patients had lower resting CECs ($p = .02$); lower resting or total CECs correlated with longer PFS ($p = 0.007$, $p = .01$); no correlation with response
mCRC, Kopetz et al. (2010) [93]	FOLFIRI + bevacizumab (5 mg/kg) every 14 days	43	IL-8, VEGF, VEGFR-2	High IL-8 correlated with shorter PFS ($p = .03$); no effect on PFS or OS
T3–4 rectal cancer, Willett et al. (2009) [94] and Duda et al. (2010) [96]	Neoadjuvant bevacizumab (5 or 10 mg/kg) + 5-FU + radiation (50.4 Gy in 28 fractions)	32	VEGF, PlGF, IL-6, IL-8, VEGFR-1, CECs post-treatment	No effect; significant association with higher pT stage ($p < .01$) and Mandard grade ($p < .05$); significant correlation with pCR ($p < .05$)
Locally advanced breast cancer, Baar et al. (2009) [115]	Neoadjuvant weekly docetaxel versus neoadjuvant docetaxel + bevacizumab (10 mg/kg every 14 days)	24 versus 25	VCAM-1, E-selectin, VEGF, ICAM-1, bFGF	Inversely predictive of response ($p = .033$ and $p = .035$) on univariate analysis but not multivariate analysis; no significant correlation with response
Advanced breast cancer, Dellapasqua et al. (2008) [104]	Cyclophosphamide + capecitabine + bevacizumab (10 mg/kg) every 14 days	46	CECs, CEPs	CECs significantly higher in patients who responded ($p = .01$); no correlation with outcome
Locally advanced breast cancer, Torrisi et al. (2008) [106]	Bevacizumab (15 mg/kg) every 21 days + capecitabine + vinorelbine + letrozole	36	CECs, CEPs	No increase in $CD31^+/VEGFR\text{-}2^+$; CECs correlated with response; correlation between baseline levels of CEPs and response ($p = .026$)
Previously treated advanced ovarian cancer, Garcia et al. (2008) [116]	Bevacizumab (10 mg/kg) every 14 days + cyclophosphamide	70	VEGF, E-selectin, thrombospondin	No association with response, PFS, or OS
Stage III–IV breast cancer, Denduluri et al. (2008) [117]	Neoadjuvant bevacizumab then bevacizumab + chemotherapy (regimens not specified)	21	VEGF, VEGFR-2, VCAM-1	No significant association with response
Previously treated advanced breast cancer, Burstein et al. (2008) [118]	Bevacizumab (10 mg/kg) every 14 days + weekly vinorelbine	56	VEGF	Low serum VEGF correlated with longer time to progression ($p = .003$)
Non-small cell lung cancer, Dowlati et al. (2008) [92]	Carboplatin + paclitaxel versus carboplatin + paclitaxel + bevacizumab (15 mg/kg) every 21 days	444 versus 434	VEGF, ICAM-1 E-selectin, bFGF	High VEGF correlated with greater response with chemotherapy plus bevacizumab than with chemotherapy alone ($p = .01$); significant inverse correlation with response ($p = .02$), OS ($p = .00005$) but not PFS; no significant correlation with PFS or OS
Metastatic breast cancer, Ramaswamy et al. (2006) [91]	Bevacizumab (10 mg/kg) every 14 days + weekly docetaxel	27	E-selectin, ICAM-1; VEGF; P-selectin, VCAM-1, FGF, PDGF, MMP-2, MMP-9	Higher in responders ($p = .02$); higher in responders ($p = .03$), but significance lost when outlying values excluded ($p = .11$); no significant correlation with response
Metastatic RCC, Yang et al. (2003) [90]	Placebo versus bevacizumab (3 mg/kg) versus bevacizumab (10 mg/kg) every 14 days	40 versus 37 versus 39	VEGF	No significant association with response or time to progression

Abbreviations: 5-FU, 5-fluorouracil; bFGF, basic fibroblast growth factor; CECs, circulating endothelial cells; CEPs, circulating endothelial progenitor cells; FOLFIRI, 5-FU, leucovorin, and irinotecan; FOLFOX, 5-FU, leucovorin, and oxaliplatin; FOLFOXIRI, 5-FU, leucovorin, oxaliplatin, and irinotecan; ICAM, intercellular cell adhesion molecule; IL, interleukin; mCRC, metastatic colorectal cancer; MMP, matrix metalloproteinase; OS, overall survival; pCR, pathologic complete response; PDGF, platelet-derived growth factor; PFS, progression-free survival; PlGF, placental growth factor; RCC, renal cell cancer; SDF, stromal-derived factor; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; XELOX, capecitabine plus oxaliplatin.

with resting and activated cells reported at five times higher levels than in healthy controls ($p < .008$) [108]. These cells are derived from vessel walls [109] and were shown to be suppressed by antiangiogenic agents but not by chemother-

apy in preclinical studies, suggesting a role for monitoring response to antiangiogenic drugs [110]. However, results from clinical studies are currently inconsistent [104–106]. We are not aware of any reports of CEC quantification in esophagogastric cancer. Difficulties associated with understanding the origins and roles of these cells, as well as the technical complexity of isolating and identifying these cells, may make the use of CECs as a biomarker challenging.

Hypertension

Development of hypertension was correlated with the RR and a longer PFS interval in a small study of patients with mCRC treated with chemotherapy plus bevacizumab ($p < .04$), suggesting a possible predictive effect [111]. Similarly, in a retrospective analysis of a phase III study of interferon-α with or without bevacizumab for patients with advanced RCC, the PFS and OS times were longer in patients treated with bevacizumab who developed grade ≥ 2 hypertension ($p < .01$), but no significant effect on the RR was observed [112]. However, this potential clinical biomarker needs to be validated in a large, prospective study.

Imaging Biomarkers

Conventional imaging with CT scans with response evaluation using conventional CT-based criteria appears not to be the optimal imaging modality for assessing response to antiangiogenic agents. Dynamic-contrast magnetic resonance imaging has been investigated as a novel method to evaluate response to antiangiogenic agents, allowing noninvasive estimation of vascular permeability and endothelial surface area. However, the available results are mostly derived from small studies, resulting in few significant results [113], and prospective evaluation within larger studies will determine the future use of this modality in clinical practice. Positron emission tomography is frequently used in the staging of esophagogastric cancers and is an effective tool for early assessment of response to neoadjuvant therapy, with metabolic response correlating with longer survival [114]. Alternative tracers to the standard 18-fluorodeoxyglucose to potentially better image tumors in patients treated with antiangiogenic agents are currently under evaluation [113]. Imaging methods may offer a promising approach for early prediction of treatment response in patients treated with antiangiogenic therapy.

Conclusions

Despite extensive international research in the field of angiogenesis, many aspects of antiangiogenic agents and how to optimally integrate them into clinical care remain poorly understood. Preclinical concerns of a rebound effect on growth after antiangiogenic agents are withdrawn have not been borne out in clinical trials, but the reason for this discrepancy is unknown. We still lack definitive evidence to determine the optimal duration of therapy and whether antiangiogenic agents are most effectively used until or beyond disease progression. Furthermore, the mechanism underlying the lack of efficacy in the adjuvant treatment of colorectal cancer is unknown.

The monotherapy activity of the TKIs sorafenib and sunitinib in RCC patients is thought to relate to the frequent inactivation of VHL, which is not a feature of esophagogastric cancer. Evaluation of the activity of TKIs in gastric cancer will need to be undertaken with correlative translational studies to define both the mechanism of activity and any subgroups of patients who may gain most benefit.

With the absence of a validated biomarker, we are currently unable to preselect patients who may benefit from antiangiogenic drugs, or predict those who will develop toxicities. Parallel prospective translational research in current trials is critical to bridge these gaps in our knowledge and, it is hoped, one day allow us to select patients most likely to benefit from these high-cost drugs that have uncommon, but potentially serious, toxicities.

Whereas antiangiogenic agents have some activity in esophagogastric cancer patients, no trial to date has reported an OS benefit. The results of the AVAGAST study demonstrated some clinical efficacy in the first-line advanced disease setting, but failure to achieve the primary endpoint of the study meant that bevacizumab will not be integrated into routine clinical care. However, ongoing studies of bevacizumab added to neoadjuvant or perioperative chemotherapy and of ramucirumab and anti-VEGFR TKIs in the second-line treatment of advanced disease may identify a future role for these agents in esophagogastric cancer.

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