Anti-Angiogenics in Gastroesophageal Cancer

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Abstract
Gastroesophageal cancer represents a leading cause of cancer death worldwide. Over the last decades, significant improvements have been made in the systemic chemotherapy of both locally advanced and metastatic gastroesophageal cancer, and human epidermal growth factor receptor 2 (HER2) has been implemented as an important target for molecular stratified treatment. Overall, however, the prognosis of advanced gastroesophageal cancer remains poor. Preclinical data clearly indicate that angiogenesis plays a pivotal role in gastroesophageal cancer driving progression and metastasis. Consequently, antiangiogenic treatment strategies have been tested in a number of clinical trials. Currently, there is a growing body of evidence that antiangiogenic treatment strategies result in improved clinical outcome in gastroesophageal cancer. Ramucirumab, a monoclonal antibody directed against vascular endothelial growth factor receptor 2 (VEGFR2), has proven efficacy in the second-line treatment of advanced gastric cancer, either given alone or in combination with paclitaxel. Combinations of platinum-based combination chemotherapy and ramucirumab in the first-line setting of advanced disease and in the perioperative setting of localized disease are underway. This review describes the role of angiogenesis in gastroesophageal cancer biology and gives a comprehensive overview on recent clinical trials with respect to antiangiogenic treatment strategies.

Keywords
Gastric cancer • Angiogenesis • Ramucirumab • Bevacizumab • Apatinib • Chemotherapy

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Introduction

Gastroesophageal cancer is a global health problem, with 1,417,000 newly diagnosed patients per year and 1,123,000 annual deaths from this diagnosis (Ferlay et al. 2015). The incidence and geographical distribution of gastroesophageal cancer vary: non-cardia gastric cancer is more prevalent in East Asia, East-Central Europe, Latin America, and Africa, whereas adenocarcinomas of the distal esophagus, the gastroesophageal junction (GEJ), and the proximal stomach are more prevalent in Western Europe, North America, and Australia (Colquhoun et al. 2015). Gastroesophageal cancers are clinically aggressive. In the Western hemisphere, most patients present with locally advanced or metastatic disease, which mandates the use of systemic chemotherapy, either perioperatively or in the palliative setting (Lordick and Janjigian 2016).

For patients with gastroesophageal cancer that is not amenable to complete resection owing to metastatic disease, palliative chemotherapy can prolong survival and improve symptoms and quality of life compared with best supportive care (BSC) alone (Wagner et al. 2006). Chemotherapy combinations comprising platinum compounds (e.g., oxaliplatin or cisplatin) and fluoropyrimidines (5-fluorouracil [5-FU], capecitabine, or S-1) are more effective than fluoropyrimidine monotherapy in the first-line setting (Lordick et al. 2014). The addition of a third chemotherapy agent – docetaxel or epirubicin – in patients with good functional and nutritional status and with uncompromised organ functions can improve disease control and tumor response rate, which translates to a modest overall survival (OS) benefit when compared with doublet therapy (Van Cutsem et al. 2006, 2015). In the second-line setting, cytotoxic monotherapy (irinotecan, docetaxel, or paclitaxel) has been established as a standard of care (Lordick 2012; Lordick et al. 2014).

Human epidermal growth factor receptor 2 (HER2) and vascular endothelial growth factor receptor 2 (VEGF-R2) are clinically validated molecular targets in the treatment of advanced-stage gastroesophageal cancer. Trastuzumab (an HER2-targeting monoclonal antibody) and ramucirumab (an anti-VEGF-R2 antibody) are now considered standard-of-care treatments for metastatic gastroesophageal cancer (Bang et al. 2010; Fuchs et al. 2014; Wilke et al. 2014), but the availability of these drugs differs among countries. A recently proposed treatment algorithm, based on national and international guidelines and on our interpretation of the latest published data, is shown in Fig. 1 (Lordick 2015; Lordick and Janjigian 2016).

This algorithm underscores the emerging role for anti-angiogenic treatment of gastroesophageal cancer in clinical practice and highlights the need to understand the pathophysiological role of angiogenesis in this disease, mechanisms of response and resistance, and potential biomarkers.

The Role of Angiogenesis in Gastroesophageal Cancer

Biological Background

The term angiogenesis describes the formation of new blood vessels from preexisting vascular structures. In tumors, angiogenesis is driven by pro-angiogenic factors derived from tumor cells and from cellular components of the surrounding tumor stroma. The process of angiogenesis has to be distinguished from vasculogenesis, which means the formation of primitive vascular structures during early embryonic development, driven by vascular precursor cells derived from the bone marrow. While angiogenesis clearly represents a hallmark of cancer and plays a prominent role in tumor progression and metastasis, the tumor-promoting role of vascular precursor cells is still controversial due to conflicting preclinical data and may only have a limited role in human solid tumors.

With respect to gastroesophageal cancer, scientific evidence suggests that angiogenesis is centrally involved in tumor growth and metastasis, as early results have indicated that tumor vascularization correlated with hematogenous metastasis and prognosis (Tanigawa et al. 1996).
The vascular endothelial growth factor (VEGF) family comprises of different members (i.e., VEGF-A to VEGF-D and placental growth factor [PlGF]). In addition, a number of VEGF-A isoforms have been described, which are generated by alternative splicing. They exert their biological activity through binding to VEGF receptors (VEGF-R1, VEGF-R2, and VEGF-R3). While VEGF-R1 and VEGF-R2 are expressed on endothelial cells (EC) and play a central role in sprouting angiogenesis, VEGF-R3 is expressed on lymphatic vascular cells and has a role in lymphangiogenesis. VEGF is expressed by tumor cells and by different cell types of the tumor stroma, like cancer-associated fibroblasts. In contrast to “normal,” physiological angiogenesis, tumor angiogenesis driven by proangiogenic factors results in the formation of a structurally abnormal blood vessel network, which causes an increase in interstitial fluid pressure within tumors and in turn a decrease in the accessibility of chemotherapeutic compounds.

In gastric cancer, high VEGF expression has predominantly been found in intestinal-type tumors correlating with vessel counts (Takahashi et al. 1996). Blood VEGF levels correlated with clinical stage and highest VEGF concentrations
were found in metastatic gastric cancer patients (Yoshikawa et al. 2000). Preclinical data showed that invasion of gastric cancer cells was driven by VEGF in an ανβ6 integrin-dependent manner (Zhao et al. 2010). The expression of VEGF-C, which plays a role in lymphangiogenesis, was associated with intestinal-type cancer and correlated with lymphatic invasion and lymph node metastasis (Onogawa et al. 2005). Finally, VEGF has been described as a prognostic marker in gastric cancer patients with advanced or metastatic disease (Van Cutsem et al. 2012).

In esophageal cancer, tumor angiogenesis is also centrally involved. VEGF expression and VEGF blood levels were increased both in squamous cell carcinoma and adenocarcinoma patients compared to healthy controls, and there was a correlation with vessel counts (Kitadai et al. 1998). Interestingly, VEGF-R1 expression in the tumor tissue was correlated with dissemination of tumor cells to the bone marrow in patients with esophageal cancer (42% adenocarcinoma) underlining a role of this pathway in metastatic progression (Schultze et al. 2012).

Platelet-Derived Growth Factor (PDGF)
Platelet-derived growth factor (PDGF) signaling is centrally involved in tumor angiogenesis. Four PDGF genes (PDGF-A to PDGF-D) are known, forming five dimeric PDGF isoforms (i.e., PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD). Signal transduction is mediated by PDGF-receptor α (PDGF-Rα) and PDGF-Rβ as well as heterodimeric PDGF-Rα/PDGF-Rβ complexes. While PDGF-AA, PDGF-AB, PDGF-BB, and PDGF-CC activate PDGF-Rα, PDGF-Rβ signaling is mediated through PDGF-BB and PDGF-DD. The heterodimeric PDGF-Rα/PDGF-Rβ complexes are activated by binding PDGF-AB, PDGF-BB, and PDGF-CC, respectively (Fredriksson et al. 2004).

PDGF-BB is abundantly expressed in fibroblasts, myofibroblasts, pericytes, and vascular smooth muscle cells (VSMC). PDGF-Rβ activation by PDGF-BB in stromal and vascular mural cells from the tumor microenvironment stimulates angiogenesis: PDGF-BB has marked effects on vascular remodeling, maturation, and stability by recruiting pericytes and VSMC to newly formed angiogenic vessels (Heldin 2012, 2013). It is believed that the responsiveness of mature vessels, which are characterized by a tight spatial interaction between EC and PC toward VEGF-targeting therapies, can be enhanced by blocking the PDGF-R-signaling cascade, thus disrupting the interaction between EC and PC, resulting in a more immature vascular state. Important interactions have been described linking VEGF-R2 activation with an inhibition of PDGF-Rβ signaling in perivascular cells. Similarly, an interaction between fibroblast growth factor-2 (FGF-2) and PDGF-BB-mediated signaling was described due to an upregulation of PDGF-Rβ upon stimulation with FGF-2 in perivascular cells (Cao et al. 2008). From this background it becomes clear that simultaneous blockade of two or more pathways represents a promising strategy to target tumor angiogenesis. In a series of 109 gastric adenocarcinoma cases, VEGF-A and PDGF-BB were simultaneously expressed at high levels in the tumor stroma of both intestinal- and diffuse-type gastric cancer, and phosphorylation of PDGF-Rβ was significantly associated with depth of invasion (Suzuki et al. 2010).

Angiopoietin: Tie2
The angiopoietin – tyrosine kinase with immunoglobulin and epidermal growth factor homology domains-1 (Tie1) and Tie2 receptor/ligand system – is centrally involved in vessel formation and maturation. It is characterized by activities complementing the VEGF system, specifically during late stages of vessel development following sprouting angiogenesis, by promoting EC survival and vessel maturation and stabilization. The vascular state in tumors is often immature and instable, and this phenotype is related to the formation of metastasis. Consequently, it becomes clear that angiopoietin/Tie2 signaling plays a pivotal role in tumor angiogenesis and metastasis (Thurston and Daly 2012).

Ang-1, which is mainly derived from pericytes, plays a role in stabilizing vessels by interacting with the Tie2 receptor on endothelial cells (EC). In contrast, the activity of Ang-2 is
related to vessel remodeling and to the generation of an immature vascular state. Consequently, a shift in the balance between Ang-1 and Ang-2 toward Ang-2 results in impaired pericyte coverage, vessel destabilization, and increased vascular permeability. Additionally, Ang-2 can directly induce sprouting angiogenesis by engaging ανβ5 integrins, and it is linked to the formation of metastases (Albini and Noonan 2012; Felcht et al. 2012; Rigamonti and De Palma 2013). Combined inhibition of VEGF and Ang-2 in a murine xenograft model resulted in improved vascular normalization. Interestingly, the effect was even better at lower doses of the anti-VEGF monoclonal antibody bevacizumab in combination with an anti-Ang-2 peptibody (L1–10) (Coutelle et al. 2015). These findings, among others, suggest that dosing of antiangiogenic compounds is an important issue.

In the clinical setting, a significant correlation was found between Ang-2 mRNA expression in the tumor tissue of gastric cancer patients and an immature vascular state. In addition, the presence of Ang-2 and VEGF was associated with an upregulation of matrix metalloproteinases (MMP) in vitro. Finally, high Ang-2 expression was associated with shorter survival times in gastric cancer patients (Etoh et al. 2001) and there was a significantly higher expression of Ang-2 in advanced stage gastric cancer compared to early-stage disease (Sun et al. 2004). Preoperative serum Ang-2 levels were correlated with lymph node metastasis (Jo et al. 2009), and pre-therapeutic plasma Ang-2 levels were prognostic for overall survival in the AVAGAST trial (Hacker et al. 2016).

**Integrins**

Integrins are heterodimeric transmembrane receptors, centrally involved in the crosstalk between cancer cells as well as between cancer cells and other cellular and noncellular components of the tumor microenvironment. Integrin-signaling pathways in tumor cells are very similar to those observed in activated EC that in principle require the same functional properties to remodel during tumor angiogenesis. Sprouting EC are characterized by expression of a unique profile of integrins. Furthermore, integrin-mediated signaling occurs in tumor-associated fibroblasts and inflammatory cells that contribute to tumor angiogenesis. Finally, pericyte coverage of maturing blood vessels is influenced by integrin adhesion to ECM proteins within the tumor stroma, linking integrin function to vascular remodeling and maturation (Weis and Cheresh 2011). In a large cohort (n = 482) of gastric cancer patients, both ανβ3 and ανβ5 integrins were expressed in at least one tumor component (i.e., tumor or stroma). Both were expressed significantly more often in intestinal-type gastric cancer, and patients positive for expression of ανβ3 on endothelial cells showed a significantly longer survival. In addition, patients with intestinal-type gastric cancer negative for expression of ανβ5 on stroma cells had significantly longer survival (Boger et al. 2015).

**Cellular Components of the Tumor Stroma that Drive Angiogenesis in Gastric Cancer**

Progression of solid tumors is driven by a crosstalk of tumor cells with surrounding cells of the tumor stroma. Among them, fibroblasts accumulate in the activated stroma. The so-called cancer associated-fibroblasts (CAF) were shown to play a pivotal role in promoting tumor growth, inflammation, angiogenesis, and metastasis in different solid tumors as well as in gastric cancer (Guo et al. 2008). In gastric cancer, the expression of galectin-1, an evolutionarily conserved glycan-binding protein with angiogenic potential, which is overexpressed in CAF, was demonstrated to be correlated with VEGF expression and CD31 expression from EC. Furthermore, high expression of galectin-1 in CAF increased proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVEC), as well as VEGF-R2 phosphorylation and enhanced VEGF expression in gastric cancer cells (Tang et al. 2016). In another translational study in gastric cancer, immunohistochemistry and mRNA expression of protein markers of CAF were used to determine their prevalence in the tumor tissue.
There was a correlation with tumor size, depth of tumor invasion, lymph node metastasis, and liver or peritoneal metastasis. Importantly, CAF-specific proteins were identified that might serve as both prognostic markers and as novel targets for anticancer drugs (Zhi et al. 2010).

Macrophages represent another important cell type of the tumor stroma, involved in tumor progression and metastasis. Primarily, they play a central role in innate host defense. Precursor cells migrate to target tissues where they mature and acquire different phenotypes. Generally, macrophages have been divided into two major subtypes, M1 and M2, based on differences in cell surface receptors and gene expression data (Mantovani et al. 2005). Tumor-associated macrophages (TAM) were demonstrated to exhibit functions of M2 macrophages and can be characterized as M2d subtype according to a recent subclassification (Murray et al. 2014). M1 macrophages secrete pro-inflammatory cytokines and are involved in MHC class I- and II-mediated presentation of tumor antigens and in the stimulation of Th1 responses such as cytotoxic T-lymphocyte (CTL) generation. Therefore, they are assumed to exert antitumoral functions. In contrast, M2 macrophages secrete immunosuppressive cytokines, such as IL-10, CCL17, and CCL22, and produce pro-angiogenic and tissue remodeling factors such as VEGF, PlGF, and matrix metalloproteinase 9 (Mantovani and Sica 2010). Macrophage polarization has been demonstrated to play a crucial role in determining the maturation status of the tumor vasculature in murine models. More specifically, the soluble factor histidine-rich glycoprotein (HRG) when over-expressed in tumor cells induced vascular normalization associated with a shift toward M1 macrophages. Moreover, tumor growth and metastasis were inhibited. Mechanistically, HRG downregulated the expression of PlGF by TAM. Interestingly, HRG levels were found to be decreased in human cancer (Rolny et al. 2011). In addition, M2 macrophage-derived PlGF and VEGF-C was shown to play an important role in inducing resistance toward VEGF-targeting drugs, which are an integral part of standard treatment in many cancer entities (Fischer et al. 2007).

In gastric cancer, there is clear evidence for a tumor-promoting role of TAM. Recently, a correlation of the frequency of M2 polarized macrophages with overall survival was demonstrated in a cohort of 180 patients with gastric cancer (Zhang et al. 2015). In another study, intraperitoneal TAM in gastric cancer patients with peritoneal dissemination were found to be polarized to the M2 phenotype (Yamaguichi et al. 2015).

**Potential Novel Targets and Drug Development**

Targeting the VEGF axis is the most commonly used antiangiogenic approach: the majority of currently used anti-angiogenic treatment strategies in different solid tumors like colorectal, breast, lung, ovarian, or cervical cancer rely on the blockade of this signaling pathway using monoclonal antibodies, bevacizumab (VEGF-A), ramucirumab (VEGF-R2), or the fusion construct aflibercept targeting VEGF-A, VEGF-B, and PlGF, respectively. Inhibition of the VEGF-pathway using tyrosin kinase inhibitors (TKI), targeting different VEGF-Rs, has proven to be efficient in renal cell cancer and thyroid cancer.

TKI can target different pathways, thus allowing combinations of targets that might result in improved antiangiogenic treatment efficacy. The TKI nintedanib targets VEGF-R, PDGF-R, and fibroblast growth factor receptors. Based on the importance of these pathways for angiogenesis and tumor cell proliferation, nintedanib is a promising compound. Recently, nintedanib has been approved for second-line treatment of non-small cell lung cancer in combination with docetaxel (Reck et al. 2014) and is going to be tested in gastroesophageal cancer patients as well in the near future.

The angiopoietin/Tie2 receptor ligand system represents another attractive drug target in gastric cancer. Both a fully human species cross-reactive Ang-2 selective antibody (LC06) and a corresponding Ang-2/-1 cross-reactive antibody (LC08) have been developed. Preclinical data indicate an increased specificity of the Ang-2 selective antibody for tumor vasculature
compared to the Ang-2/Ang-1 reactive antibody, which additionally induced regression of physiological vessels (i.e., in the trachea) (Thomas et al. 2013). Trebananib (formerly AMG 386) is a peptide-Fc fusion protein (i.e., peptibody) that inhibits angiogenesis by preventing the interaction of Ang-1 and Ang-2 with their receptor, Tie2 (Herbst et al. 2009). The compound is in late clinical development; however, a recent phase III study in ovarian cancer failed to demonstrate an overall survival benefit (Sheridan 2015), and in a phase II study in gastroesophageal cancer patients, there was no benefit for adding trebananib to chemotherapy (Eatock et al. 2013). These data might indicate that a combined inhibition of both Ang-1 and Ang-2 could be less effective than blocking Ang-2 alone. As an alternative combination of Ang-2/Tie2 inhibition with inhibitors of well-characterized targets like VEGF-R-signaling is attractive, since combined antiangiogenic treatment can prevent primary or secondary resistance in preclinical models, which are mediated by the upregulation of alternative angiogenic pathways (Rigamonti et al. 2014).

In this respect, recent technological advances enabled the development of bispecific antibodies and antibody constructs termed CovX-Bodies. These constructs consist of two different peptide pharmacophores covalently bound to the nucleophilic heavy chain lysine at position 93 located deep in the hydrophobic binding pockets on each of the two Fab arms of the scaffold antibody (Doppalapudi et al. 2010). While the antibody scaffold enables long half-life times and distribution properties very similar to IgG, the peptide pharmacophores of the CovX-Body are responsible for functional activities.

Different CovX-Bodies targeting Ang-2 were generated and extensively tested in murine models. Interestingly, these compounds were shown to reduce the frequency of pro-angiogenic TEM in the tumor stroma as well (Huang et al. 2011). The CovX-Body (CVX-060) showed good pharmacodynamic properties (half-life 110h) and efficacy. Currently this compound is being tested in early clinical trials in combination with angiogenesis-targeting TKI. Additionally, a CovX-Body that targets both Ang-1 and VEGF has been developed that showed favorable antiangiogenic activity in murine models and is now further developed for clinical testing (Kienast et al. 2013; Thomas et al. 2012).

Although integrins play a key role in tumor cell-tumor cell and tumor cell-stroma cell interactions as well as in propagating tumor angiogenesis, recent efforts to target integrins have not been successful. In principle, integrin ligand binding can be mimicked with synthetic peptides containing the RGD sequence such as cyclic RGD peptides that competitively inhibit ligand binding to integrins, thus disrupting integrin-mediated signaling pathways. RGD-mimetic peptides or small molecules act as potent antiangiogenic compounds by disrupting $\alpha_v\beta_3/\alpha_v\beta_5$ integrin-ligand interactions. Such compounds were shown to inhibit angiogenesis in preclinical models by blocking the proliferation of EC and by inducing apoptosis of EC. Cilengitide was the first small molecule cyclic peptide targeting the integrins $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_5\beta_1$ that was developed for clinical application (Mas-Moruno et al. 2010). There was a clear focus on the treatment of malignant highly vascularized brain tumors (i.e., glioblastoma). Recently, however, a placebo-controlled randomized phase III clinical trial failed to demonstrate a benefit for the use of cilengitide combined with standard treatment in patients with glioblastoma (Tucci et al. 2014; Stupp et al. 2014; Kurozumi et al. 2012). Thus, it is unclear today, whether this class of drugs will indeed make its way to the clinic. In this respect dosing aspects again might play an important role, as low doses of cilengitide were shown to mediate pro-angiogenic activity. Another very interesting recent finding is that interventions improving angiogenesis with respect to increasing the number of functional blood vessels in tumors using a low-dose therapy regimen of cilengitide and verapamil enhanced the uptake of chemotherapy (gemcitabine), improved tumor metabolism, and resulted in reduced tumor growth and progression in murine models (Wong et al. 2015).

Besides targeting specific angiogenic pathways or pathway combinations, another attractive approach is to target cell types that are centrally involved in angiogenesis like TAM or CAF.
Based on the fact that colony stimulating factor 1 (CSF-1) represents a major survival factor for macrophages in the tumor microenvironment, a monoclonal antibody against CSF-Receptor-1 (CSF-R1) was generated, which led to a depletion of TAM and showed antitumor activity in preclinical models and in patients with giant cell tumors (Ries et al. 2014). Another approach is to shift the polarization of TAM from angiogenic M2 type toward the antitumoral M1 type. However, while basic mechanisms of TAM polarization have been described in preclinical models (Rolny et al. 2011), no specific pharmacological approach is clinically available, yet. With respect to CAF targeting, a number of immunological strategies (i.e., vaccines, T-cell therapies) have been tested (Kakarla et al. 2012). Another preclinical study demonstrated that inhibition of the PDGF-signaling axis resulted in suppression of pro-tumoral activities of CAF (Pietras et al. 2008). In this respect TKI targeting PDGF-R signaling together with other pathways like nilotinib (VEGF-R, PDGF-R, FGF-R) are promising.

Angiogenesis-Related Biomarker Research and Gastric Cancer

Over the last decade, intense efforts have been made to identify biomarkers predicting the efficacy of antiangiogenic drugs. The largest database has been generated with respect to the anti-VEGF antibody bevacizumab, which was the first antiangiogenic compound to enter clinical research focused on expression levels of VEGF in tumor tissue and on the measurement of VEGF in the blood (serum, plasma). Furthermore, single nucleotide polymorphisms (SNP) in angiogenesis-related genes (i.e., VEGF and VEGF receptors) were extensively studied in different cohorts. While in some studies predictive markers could be identified, overall, the results remained inconclusive and often could not be reproduced (Maru et al. 2013; Jubb and Harris 2010; Lambrechts et al. 2012). In a cohort of patients with locally advanced or metastatic gastric cancer treated in the phase III AVAGAST trial (cisplatin/fluoropyrimidine±bevacizumab), patients with high baseline plasma VEGF-A levels showed a trend toward improved overall survival with the addition of bevacizumab (hazard ratio [HR], 0.72; 95% CI, 0.57–0.93) compared to patients with low VEGF-A levels (HR, 1.01; 95% CI, 0.77–1.31; interaction \( p = 0.07 \)). This correlation was predominantly found in the non-Asian patient population (Van Cutsem et al. 2012).

Based on the preclinical findings that resistance toward VEGF-targeting therapy can be mediated by increased expression of Ang-2 (Rigamonti et al. 2014), the prognostic and predictive role of baseline plasma Ang-2 levels were studied in the AVAGAST trial cohort. Ang-2 did not predict efficacy of bevacizumab therapy, neither alone nor in combination with baseline plasma VEGF levels. However, Ang-2 was prognostic for overall survival, and a strong correlation was found with the occurrence of liver metastasis (Hacker et al. 2016).

Clinical Results on Angiogenesis Inhibitors in Gastroesophageal Cancer

Antibodies

Targeting VEGF with Bevacizumab

Bevacizumab is a humanized monoclonal antibody that inhibits angiogenesis by neutralizing circulating VEGF. It binds to VEGF A, preventing its binding with VEGF-R1 and VEGF-R2 on the surface of endothelial cells. Bevacizumab is currently approved for the treatment of colon, lung, breast, ovarian, endometrial, and clear cell renal carcinoma in a metastatic setting. Several phase II and three completed phase III clinical trials have investigated the efficacy of first-line bevacizumab combined with chemotherapy, in patients with locally advanced or metastatic gastroesophageal cancers.

A multicenter phase II study evaluated the efficacy and safety of the addition of bevacizumab and cisplatin-irinotecan in 47 patients with advanced gastroesophageal cancers. The overall response rate (ORR) was 65%, median time to
progression (TTP) was 8.3 months, and the OS was 12.3 months.

Bevacizumab related grade 3/4 toxicity included arterial hypertension (28%), thromboembolic events (25%), gastric perforation (4.2%), and serious cardiovascular events (2.1%) (Shah et al. 2006).

In another phase II trial, bevacizumab was combined with a modified schedule of docetaxel, cisplatin, and fluorouracil in 44 patients with advanced gastroesophageal cancer. ORR was 67%, the median progression-free survival (PFS) was 12 months, and OS was 16.8 months. Although no evidence of increased chemotherapy-related toxicities with the addition of bevacizumab were shown, 39% of patients experienced venous thromboembolism (Shah et al. 2011).

Another phase II study with promising results was reported where bevacizumab was administered in combination with docetaxel and oxaliplatin in 38 patients with advanced gastroesophageal cancer. The disease control rate was 79% with a PFS of 6.6 months and an OS of 11.1 months (El-Rayes et al. 2010).

Finally, a phase II study investigated the combination of bevacizumab with capecitabine and oxaliplatin (ORR 51%, PFS 7.2 months, OS 10.8 months) (Uronis et al. 2013).

Based on these results, the international, randomized, double-blind, placebo-controlled phase III Avastin for Advanced Gastric Cancer Trial (AVAGAST) was conducted. AVAGAST investigated the combination of bevacizumab with cisplatin and capecitabine in previously untreated locally advanced or metastatic gastroesophageal cancers (Ohtsu et al. 2011). The primary endpoint of this study was OS. Seven hundred and seventy-four patients were enrolled and were 1:1 assigned to each treatment group. Median OS was 12.1 months in the bevacizumab arm and 10.1 months with placebo. However, this numerical difference did not meet the prespecified criteria for statistical significance (HR = 0.87, 95% CI, 0.73–1.03, P = 0.1002). In contrast, both PFS (median 6.7 vs. 5.3 months) and ORR (46.0% vs. 37.4%) were significantly improved with bevacizumab versus placebo. The incidence of grade ≥3 adverse events was not increased with the exception of arterial hypertension (6% vs. <1%). Interestingly, a preplanned subgroup analyses revealed regional differences in efficacy outcomes. The effectiveness of bevacizumab on all study outcomes was substantially higher among patients recruited in North and South America compared to patients recruited in Europe (intermediate effect) or in the Asia-Pacific region (no or limited effect). Different patient selection, clinical practice, and tumor and population genetics and the influence of second-line chemotherapy were discussed to explain these results (Roviello et al. 2016).

Subsequently, a smaller phase III study, with similar design as AVAGAST, was conducted in 202 Chinese patients. In the bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR) (Shen et al. 2015), the baseline patient clinical characteristics and the second line of treatments were more similar to the European-American subgroup of the AVAGAST trial. However, this study showed no significant differences between the two arms with regard to OS (10.5 months vs. 11.4 months) and PFS (6.3 months vs. 6.0 months).

Finally, ST03 was a multicenter, randomized, phase II/III study comparing perioperative epirubicin, cisplatin, and capecitabine with or without bevacizumab. The first 200 patients contributed to a phase II study powered to exclude unacceptable rates of gastrointestinal and cardiac adverse events. The incidence of cardiac complications was similar in both arms except for arterial thromboembolic events and asymptomatic drops in left ventricular ejection fraction with bevacizumab (Okines et al. 2013). Recently, survival results have been reported for ST03 in abstract form without showing any gain in OS by the addition of bevacizumab to perioperative chemotherapy (Cunningham et al. 2015).

Based on the reported results, bevacizumab is currently not an option for patients with locally advanced or metastatic gastroesophageal cancer. The results of phase III trials with bevacizumab in gastroesophageal cancer are summarized in Table 1.
Targeting VEGF-R2 with Ramucirumab

Ramucirumab, a human IgG1 monoclonal antibody directed against VEGF-R2, prevents binding of ligands to VEGF-R2 and receptor-mediated pathway activation in endothelial cells. It was shown that ramucirumab can be safely administered and that objective antitumor activity and anti-angiogenic effects are observed over a wide range of dose levels in different malignancies treated in phase I. Four (15%) of 27 patients with measurable disease had a partial response, and 11 (30%) of 37 patients had either a partial response or stable disease lasting at least 6 months. Tumor perfusion and vascularity decreased in 69% of evaluable patients. Ramucirumab 8 mg/kg intravenously given every 2 weeks was chosen as the preferred regimen for further investigation in phase III (Spratlin et al. 2010).

Anti-VEGF-R2 therapy is the first biological strategy in an unselected patient population to be associated with a survival benefit in patients with chemotherapy-refractory gastroesophageal cancer. Recently, two phase III clinical trials have showed that ramucirumab is a valuable therapeutic option in second line. Results are shown in Table 2.

Ramucirumab monotherapy for previously treated advanced gastric or gastroesophageal junction adenocarcinoma (REGARD) is an international, randomized, double-blind, placebo-controlled, phase III trial. REGARD involved patients with advanced gastroesophageal adenocarcinoma and disease progression after first-line platinum- or fluoropyrimidine-containing chemotherapy (Fuchs et al. 2014). Patients were randomly assigned (2:1) to receive best supportive care plus either ramucirumab or placebo. The primary endpoint was OS. Three hundred and fifty-five patients were assigned to receive ramucirumab (n = 238) or placebo (n = 117). Median OS was 5.2 months in the ramucirumab group and 3.8 months in the placebo group. PFS with ramucirumab was 2.1 months versus 1.3 months with placebo (HR 0.483, P < 0.0001); the rate of disease control was significantly higher in patients given ramucirumab (49% vs. 23%, P < 0.0001). Ramucirumab was well tolerated. Grade 3/4 hypertension was more common in the ramucirumab group (16% vs. 8%), whereas other adverse events were similar between groups (94% vs. 88%). Performance status was maintained for a significantly longer time with ramucirumab. Patients who received at least

Table 1 Randomized phase III studies investigating bevacizumab in gastroesophageal cancer

<table>
<thead>
<tr>
<th>Study</th>
<th>Indication</th>
<th>Patient number (n)</th>
<th>Regimen</th>
<th>Primary endpoint</th>
<th>Overall response rate</th>
<th>Progression-free survival (mon)</th>
<th>Overall survival (mon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVAGAST Ohtsu et al. (2011)</td>
<td>Stage IV 1°</td>
<td>674</td>
<td>CX+Bev CX +placebo</td>
<td>OS</td>
<td>46% versus 37.4% (HR = 8.61; 95% CI, 0.6–16.6; P = 0.0315)</td>
<td>6.7 versus 5.3 (HR = 0.80; 95% CI, 0.68–0.93; P = 0.0037)</td>
<td>12.1 versus 10.1 (HR = 0.87; 95% CI, 0.73–1.03; P = 0.1002)</td>
</tr>
<tr>
<td>AVATAR Shen et al. (2015)</td>
<td>Stage IV 1°</td>
<td>202</td>
<td>CX+Bev CX +placebo</td>
<td>OS</td>
<td>41% versus 34% (HR = 7.02; 95% CI, −8.3–22.4; P = 0.34)</td>
<td>6.3 versus 6.0 (HR = 0.89; 95% CI, 0.66–1.21; P = 0.47)</td>
<td>10.5 versus 11.4 (HR = 1.11; 95% CI, 0.79–1.56; P = 0.56)</td>
</tr>
<tr>
<td>STO3 Cunningham et al. (2015)</td>
<td>Stage Ib-III</td>
<td>1063</td>
<td>ECX+Bev ECX</td>
<td>OS</td>
<td>40% versus 42%; no statistical comparison</td>
<td>Not reported</td>
<td>34.5 versus 34.0 (HR 1.067; 95% CI 0.8911–1.279; p = 0.4784)</td>
</tr>
</tbody>
</table>

Bev bevacizumab, CX cisplatin+capecitabine, ECX epirubicin+cisplatin+capecitabine, mon months, n number, OS overall survival
four cycles of ramucirumab maintained their quality of life. Although no regional differences in the effects of ramucirumab were reported, the small number of Asian patients (16%) recruited for REGARD does not allow for any definitive conclusion.

Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastroesophageal junction adenocarcinoma (RAINBOW) is a randomized, placebo-controlled, double-blind, phase III trial. Patients had advanced gastroesophageal adenocarcinoma and disease progression on or within 4 months after first-line chemotherapy (platinum plus fluoropyrimidine with or without an anthracycline) (Wilke et al. 2014). The primary endpoint was OS. Six hundred and sixty-five patients were randomly assigned (1:1) to treatment with paclitaxel plus ramucirumab or placebo. OS was significantly longer in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (median 9.6 vs. 7.26 months). The study also met its secondary endpoint of PFS (2.86 vs. 2.86 months, HR = 0.635; 95% CI, 0.536–0.752; P = 0.0001) and ORR (16% vs. 10% at 9 months). Subgroup analyses suggest that ramucirumab has similar activity in both Asian (41.6% of the study population) and non-Asian patients. Overall, ramucirumab was well tolerated, although adverse events of grade ≥3 were somewhat greater in the combination arm and included neutropenia (40.7% vs. 18.8%). But the incidence of febrile neutropenia was similar (3.1% vs. 2.4%). Arterial hypertension (14.1% vs. 2.4%) and fatigue (7.0% vs. 4.0%) were more common with ramucirumab (Wilke et al. 2014). A subsequent analysis showed that ramucirumab in combination with paclitaxel prolonged overall survival while maintaining patient quality of life with delayed symptom worsening and functional status deterioration (Al-Batran et al. 2016).

Based on these results, ramucirumab has been granted approval by the US Food and Drug Association (FDA) and the European Medical Agency (EMA) as second-line treatment in patients with advanced or metastatic gastroesophageal cancers who progressed on fluoropyrimidine- or platinum-containing first-line chemotherapy. However, based on economic considerations, ramucirumab is not refunded in all health systems. A biomarker-based selection of patients who have a greater benefit from antiangiogenic treatment would probably help to convince authorities.

In contrast, ramucirumab failed to show superiority over chemotherapy alone in the first-line setting. In a double-blind phase II trial, 168 patients with previously untreated, unresectable locally advanced or metastatic gastroesophageal adenocarcinoma were randomized to receive modified FOLFOX6 plus ramucirumab or placebo (Yoon et al. 2014). PFS which was the primary endpoint and OS were similar in both arms (median PFS 6.4 vs. 6.7 months and median OS 11.7 vs. 11.5 months). Subgroup analyses suggest that the inclusion of patients with esophageal cancers (>45%) and a higher rate of

### Table 2

<table>
<thead>
<tr>
<th>Study</th>
<th>Indication</th>
<th>Patient number (n)</th>
<th>Regimen</th>
<th>Primary endpoint</th>
<th>Overall response rate</th>
<th>Progression-free survival (mon)</th>
<th>Overall survival (mon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>REGARD</td>
<td>Stage IV 2†</td>
<td>355</td>
<td>Ram + BSC versus BSC</td>
<td>OS</td>
<td>3% versus 3% (P = 0.76)</td>
<td>2.1 versus 1.3 (HR = 0.483; 95% CI, 0.376–0.620; P = 0.0001)</td>
<td>5.2 versus 3.8 (HR = 0.776; 95% CI, 0.603–0.998; P = 0.047)</td>
</tr>
<tr>
<td>RAINBOW</td>
<td>Stage IV 2†</td>
<td>665</td>
<td>Pac + Ram versus Pac + placebo</td>
<td>OS</td>
<td>28% versus 16% (P = 0.0001)</td>
<td>4.40 versus 2.86 (HR = 0.635; 95% CI, 0.536–0.752; P = 0.0001)</td>
<td>9.63 versus 7.26 (HR = 0.807; 95% CI, 0.678–0.962; P = 0.0169)</td>
</tr>
</tbody>
</table>

*BSC best supportive care, **mon** months, **n** number, **OS** overall survival, **Pac** paclitaxel, **Ram** ramucirumab
treatment discontinuation before progression in the investigational arm (27% vs. 10%) may have negatively impacted on the results of the study.

The phase III study of ramucirumab (LY3009806) in combination with capecitabine and cisplatin in participants with stomach cancer (RAINFALL) is now recruiting 616 patients with metastatic HER2-negative gastric cancer to receive cisplatin/capecitabine chemotherapy with or without ramucirumab in first line (NCT trial number 02314117).

Tyrosine Kinase Inhibitors

Multitarget TKI represent another approach to block angiogenesis by simultaneously targeting VEGF-R and other signaling pathways.

Sunitinib

Sunitinib, an oral inhibitor of multiple kinases, has shown broad effects in different solid tumors, and its activity is mediated through platelet-derived growth factor receptor (PDGF-R), VEGF-R, KIT, Flt-3, and RET that impair tumor proliferation and angiogenesis (O’Farrell et al. 2003). In a phase II study, sunitinib at 50 mg/day for 4 weeks, followed by 2 weeks off treatment, was given to 78 patients with advanced gastroesophageal adenocarcinoma who had failed prior chemotherapy. Two patients (2.6%) had partial responses, and 25 patients (32.1%) maintained stable disease for at least 6 weeks. Median PFS was 2.3 months (95% CI, 1.6–2.6 months), and median OS was 6.8 months (95% CI, 4.4–9.6 months). Grade ≥3 thrombocytopenia and neutropenia were reported in 34.6% and 29.4% of patients, respectively, and the most common non-hematologic adverse events were fatigue, anorexia, nausea, diarrhea, and oral mucositis (Bang et al. 2011). Similar results were observed in another phase II study that enrolled 52 pretreated patients to receive sunitinib 50 mg/day for 4 weeks with 2 weeks rest until disease progression or unacceptable toxicity. The ORR was only 3.9%, median PFS was 1.3 months (95% CI, 1.18–1.90), and median OS was 5.8 months (95% CI, 3.48–12.32). Serious adverse events occurred in 26 patients, leading to 13 treatment-related deaths (Moehler et al. 2011). Due to the lack of clinically relevant activity of sunitinib as single agent in advanced gastric cancer, its role has been assessed in combination with chemotherapy. A Korean study randomized 107 patients with unresectable or metastatic gastric cancer to single-agent docetaxel (60 mg/m², every 3 weeks) or docetaxel (60 mg/m² every 3 weeks) in combination with sunitinib (37.5 mg/day). The primary endpoint was TTP which was not significantly prolonged in the combination arm when compared with the chemotherapy alone arm: 3.9 months (95% CI 2.9–4.9) versus 2.6 months (95% CI 1.8–3.5), with an HR of 0.77 (95% CI 0.52–1.16, p = 0.21). Patients exposed to the combination experienced more stomatitis, diarrhea, and hand-foot syndrome (Yi et al. 2012). A phase I study evaluated the maximum tolerated dose (MTD), safety, pharmacokinetics, and antitumor activity of sunitinib plus S-1 and cisplatin in patients with metastatic gastric cancer. The oral angiogenic inhibitor was administered on a continuous daily dosing or with a 2-weeks-on/2-weeks-off schedule (25–37.5 mg/day), plus S-1 (80–120 mg/day) and cisplatin (60 mg/m²). MTD of sunitinib was 25 mg/day; this regimen had a manageable safety profile and promising antitumor activity. The most frequently reported ≥grade 3 adverse events were neutropenia (93.8%) and leucopenia (75.0%). The ORR was 37.5%; six additional patients did not have any disease progression for ≥24 weeks. Median PFS was 12.5 months. No pharmacokinetic interactions were observed between sunitinib and S-1 or cisplatin (Boku et al. 2014).

Sorafenib

Sorafenib is a TKI that targets VEGF-R2, PDGF-R, RET, FLT3, and RAF and interferes with tumor growth, progression, and angiogenesis. A phase I study demonstrated acceptable toxicity and preliminary efficacy when combining sorafenib (400 mg bid, days 1–35) with S-1 (40 mg/m² bid, days 1–21) and cisplatin (60 mg/m², day 8). Thirteen patients were enrolled and received at least one dose of the study treatment. No specific or serious adverse events were reported. Five
patients had partial response, and eight had stable disease as best response (Yamada et al. 2014). In another dose-finding study of sorafenib in combination with capecitabine and cisplatin as first-line treatment in patients with advanced gastric cancer, sorafenib 400 mg twice daily, capecitabine 800 mg/m² twice daily (days 1–14), and cisplatin 60 mg/m² (day 1) were the recommended phase II doses found. An ORR of 62.5%, a median PFS of 10.0 months (95% CI, 7.4–13.8), and a median OS of 14.7 months (95% CI, 12.0–20.0) were reported (Kim et al. 2012). A phase II study was subsequently conducted to determine the activity and toxicity of the three-weekly combination of sorafenib (400 mg twice daily continuously), docetaxel (75 mg/m² day 1), and cisplatin (75 mg/m² on day 1) in 44 patients with advanced gastric cancer. Partial response, the primary endpoint, was reported in 41% (18 patients), and 32% (14 patients) achieved stable disease. A median PFS of 5.8 months and a median OS of 13.6 months were reported (Sun et al. 2010).

Regorafenib
Regorafenib, which targets several receptor tyrosine kinases, including VEGF-R2, also showed enhanced antitumor activity compared with placebo in a randomized phase II study in patients with gastroesophageal cancer after failure of first-line or second-line chemotherapy (Pavlakis et al. 2016). A total of 152 patients were randomly assigned, yielding 147 evaluable patients (regorafenib, n = 97; placebo, n = 50). Median PFS significantly differed between groups (regorafenib, 2.6 months; 95% CI, 1.8–3.1 and placebo, 0.9 months; 95% CI, 0.9–0.9; HR, 0.40; 95% CI, 0.28–0.59; P < 0.001). The effect was greater in South Korean patients compared with patients enrolled in Australia, New Zealand, and Canada (HR, 0.12 v 0.61; interaction P < 0.001) but was consistent across age, neutrophil-to-lymphocyte ratio, primary site, lines of chemotherapy, peritoneal metastasis presence, number of metastatic sites, and plasma vascular endothelial growth factor A. A survival trend in favor of regorafenib was seen. Twenty-nine patients assigned to placebo received open-label regorafenib after disease progression. Regorafenib toxicity was similar to that previously reported (Pavlakis et al. 2016). The next step for the investigators will be to consider the design for a phase III trial and seek funds for this.

Apatinib
Apatinib (YN968D1) is a novel, highly potent VEGF-R2 inhibitor with a binding affinity ten times that of sorafenib (Tian et al. 2011). Based on the results of a previous phase I trial showing activity in Chinese patients with metastatic gastric cancer, a phase II randomized, double-blind, placebo-controlled trial was conducted to test this new drug in pretreated gastric cancer patients. The aim of this study was to assess the activity and safety of daily administration of third-line apatinib and to compare the tolerability of a once-daily or a twice-daily regimen. One hundred and forty-four patients were randomly assigned to receive placebo (arm A), apatinib 850 mg once daily (arm B), or apatinib 425 mg twice daily (arm C). The median OS was 2.5 months for arm A (95% CI, 1.87–3.70), 4.8 months for arm B (95% CI, 4.03–5.97), and 4.3 months for arm C (95% CI, 3.83–4.77); the median PFS was 1.4 months (95% CI, 1.20–1.83), 3.7 months (95% CI, 2.17–6.80), and 3.2 months (95% CI, 2.37–4.53), respectively. Both median PFS (p < 0.001) and median OS (p < 0.001) were statistically longer in the groups exposed to apatinib, and nine patients had a partial response. Toxicities were overall tolerable and easily clinically managed. The most common grade 3–4 adverse events were hand–foot syndrome and hypertension, while severe hematologic toxicities were rare (Li et al. 2013). A recently published randomized, double-blind, placebo-controlled phase III trial of apatinib in patients with chemotherapy-refractory advanced or metastatic gastroesophageal adenocarcinoma assessed the efficacy and safety of apatinib in patients with advanced gastric or gastroesophageal junction adenocarcinoma for whom at least two lines of prior chemotherapy had failed (Li et al. 2016). OS was the primary endpoint. Two hundred and sixty-seven patients were enrolled and were randomly assigned to oral apatinib 850 mg or placebo once daily. Median OS was significantly improved in
the apatinib group compared with the placebo group (6.5 months; 95% CI, 4.8–7.6 vs. 4.7 months; 95% CI, 3.6–5.4; \( P = 0.0149 \); hazard ratio, 0.709; 95% CI, 0.537–0.937; \( P = 0.0156 \)). Similarly, apatinib significantly prolonged PFS compared with placebo (2.6 months; 95% CI, 2.0–2.9 vs. 1.8 months; 95% CI, 1.4–1.9; \( P < 0.001 \); hazard ratio, 0.444; 95% CI, 0.331–0.595; \( P < 0.001 \)). The most common grade 3–4 non-hematologic adverse events were hand–foot syndrome, proteinuria, and hypertension.

**Future Directions**

While the benefit of systemic chemotherapy in locally advanced and metastatic gastroesophageal cancer has reached a plateau, the possibility of treating gastroesophageal cancer patients with novel drugs has recently emerged. Antiangiogenic compounds are among the first effective novel drugs in this disease. Recently published studies from The Cancer Genome Atlas (TCGA) (Cancer Genome Atlas Research 2014) and the Asian Cancer Research Group (ACRG) (Cristescu et al. 2015) proposed a novel gastric cancer classification based on different molecular features. These may serve as a roadmap for future drug development and exploration of novel drug targets. The role of new antiangiogenic drugs may be of particular relevance in specific subtypes, in which angiogenic pathways are upregulated. Nonetheless, disease anatomy and classical biology parameters remain important and should be integrated with novel molecular classifications. Understanding and targeting the mechanisms of resistance to antiangiogenic drugs is a key point in order to improve the outcome for patients with advanced gastroesophageal cancer. Mechanisms of resistance can be VEGF axis dependent, stromal dependent, or associated with non-VEGF modulators (Jayson et al. 2016). Overcoming such mechanisms will ensure a better use and results of most novel anti-angiogenic drugs. Beyond that, a crucial issue in further development of anti-angiogenic drugs is the search for predictive biomarker tests that predict which patients will, and will not, benefit before initiation of therapy. Development of biomarkers is important because of the need to balance efficacy, toxicity, and cost. Novel combinations of these drugs with other anti-angiogenics or other classes of drugs are being developed, and the appreciation that these drugs have immunomodulatory and other modes of action will eventually lead to combination regimens that are based on these newly understood mechanisms.

**Cross-References**

- Anti-Angiogenic Targets: Angiopoietin and Angiopoietin-Receptors
- Anti-Angiogenic Targets: VEGF and VEGF-Receptors
- Biomarkers for Anti-Angiogenic Therapy
- Mechanisms of Anti-Angiogenic Therapy
- Mechanisms of Tumor Angiogenesis
- Pathology of Tumor Angiogenesis

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