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Angiogenic inhibitors in gastric cancers and gastroesophageal junction carcinomas: A critical insight

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Contents

Abstract

Advanced gastric cancer ranks second as the global leading cause of cancer-related death and improvements in systemic chemotherapy have reached a plateau. Advanced molecular sequencing techniques help identifying patients more likely to respond to targeted agents; nevertheless we are still far from major breakthroughs. Although antiangiogenic drugs have produced notable advances, redundant pathways or mechanisms of resistance may limit their efficacy. Novel compounds have been recently developed to specifically target VEGF receptors, PlGF, FGF, MET, and angiopoietin. Ramucirumab, a monoclonal antibody specifically directed against the VEGFR-2, has emerged as a novel therapeutic opportunity. REGARD and RAINBOW were the first phase III studies to report the value of this strategy in gastric cancer patients, and other ongoing trials are testing novel antiangiogenic compounds. The aim of our review is to present the state-of-the-art of novel antiangiogenic compounds in advanced gastric cancer, underlying the biology, their mechanism of action, and their clinical results.

Keywords: Angiogenesis; Advanced gastric cancer; Bevacizumab; Ramucirumab

1. Introduction

Gastric cancer (GC) is one of the most common cancers worldwide and represents a public health concern [1], with a rise in incidence and mortality for proximal

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location close to the gastroesophageal junction (GEJ) [2]. Despite the improvements achieved for patients with early stage disease [3,4], the 5-year survival rate for those with metastatic disease remains vastly disappointing and median overall survival (OS) is limited to 12 months [5]. Standard first-line chemotherapy regimens for metastatic disease may vary across different countries. Nevertheless, numerous global trials confirmed that the use of a platinum- and fluoropyrimidine-based regimen may enhance patients' outcome along with quality of life [6–8]; similarly, three different randomized trials showed improved outcomes for patients receiving single-agent irinotecan or a taxane as second-line compared to best supportive care alone [9–11]. Recently, novel targeted therapies have emerged as a new hope for GC management. While EGFR-inhibitors [12,13] and mTOR-inhibitors [14] failed to provide any benefit, the addition of trastuzumab produced significant survival improvements in patients with HER-2 positive gastric or GEJ cancers (median survival increase of 3 months, hazard ratio (HR) 0.74, 95% CI 0.60–0.91, $p = 0.0046$) [15]. However, this therapeutic option is available for few potential candidates, as the HER2 overexpression (IHC) or amplification (FISH) is identified in less than 20% of patients. In gastrointestinal malignancies, angiogenesis is a well-known underlying promoter of tumor growth, invasion, and metastases. Based on a solid biologic background [16–18], the role of antiangiogenic drugs has been extensively investigated in gastric cancers [19]. Among many other molecules, bevacizumab, sorafenib, and sunitinib have been tested in clinical trials. Recently, ramucirumab has been reported as the first antiangiogenic drug to improve survival in pretreated patients with advanced GC. We reviewed published literature and ongoing trials through PubMed and Clinicaltrials.gov, respectively. The aims of this review are to define the biologic importance of angiogenesis in both gastric and gastroesophageal cancer, to critically recall the steps of the development of antiangiogenic therapy for patients with such diseases and to report the results of recent phase II and phase III randomized trials discussing the potential future role of these new agents in clinical practice.

2. The biology of angiogenesis in gastric and gastroesophageal carcinomas

Angiogenesisis a key process of the tumor growth as it ensures oxygen and nutrients supply to proliferating tumor cells. The increase of tumor size and the functional abnormalities of tumor vasculature, however, result in hypoxia and necrosis of tumor center [20–22]. Tissue hypoxia is a powerful inducer of VEGF expression [23] which, in turn, stimulates angiogenesis, invasive tumor growth and metastasis [24]. VEGF family comprises five distinct VEGF members including VEGF-A, placental growth factor (PlGF), VEGF-B, VEGF-C, and VEGF-D. Each of these ligands interacts with its specific receptor: VEGF-A binds VEGFR-1 and VEGFR-2 [25]; PlGF and VEGF-B bind VEGFR-1, although they seem to play a secondary role in the regulation of angiogenesis as compared to VEGF-A [26,27]. VEGF-C and VEGF-D bind to both VEGFR-2 and VEGFR-3, and are involved in the regulation of lymphangiogenesis [28]. The major signal transducer in angiogenesis is VEGFR-2 [29], which regulates endothelial cell proliferation through a number of different pathways (Fig. 1) [23,30]. The VEGFA-VEGFR2 binding triggers the receptor dimerization and the autophosphorylation of the VEGFR2 tyrosine-kinase site, which leads to the hydrolysis of phosphatidylinositol 4,5 bisphosphate. This cascade process generates inositol-3,4,5 trisphosphate and diacylglycerol, which eventually activate different downstream signaling cascades such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), p38 kinase, and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), all belonging to the mitogen-activated protein kinases (MAPKs) family [31]. VEGFA/VEGFR-2 interaction regulates proliferation, migration, permeability, invasion, and tube formation of endothelial cells. Disrupting this circle with the use of antiangiogenic drugs that block VEGFs and VEGFR-2 is therefore considered a key treatment modality in gastric cancer patients.

There are many mechanisms which guarantee an adequate blood supply to the tumor, including sprouting and intussusceptive angiogenesis, recruitment of endothelial progenitor cells, vessel co-option, vasculogenic mimicry and lymphangiogenesis [32]. A new form of neoangiogenesis in gastric cancer called the "cavity type" has been recently described, but its clinical relevance is unclear [33]. While the clinical efficacy of antiangiogenic therapy has been clearly shown in some type of solid tumors, it is still unknown whether this benefits maintained over the time. Resistance to antiangiogenic therapy is multifactorial and depends on several mechanisms including (1) switch to different angiogenic factors such as fibroblast growth factor (FGF) [34], (2) recruiting of tumor vessels via VEGF-independent mechanisms, (3) differentiation of cancer stem cells into endothelial cells, (4) development of cytogenetic abnormalities in tumor endothelial cells [35]. Differently from normal vessels, tumor capillaries are tortuous, blunt-end and chaotic in their organization causing a patchy and frequently reduced delivery of cytotoxic drugs to cancer cells [36]. As a result, blood flow does not reach each region of the tumor equally, starting from the center to the periphery [37] and the relative endothelial cell area is therefore high in the invasive tumor front, medium in tumor parenchyma and reduced in the inner portions [38]. Notably, the blood flow abnormalities also limit the access of immune effector cells in tumors [39,40]. Additionally, hypoxia induces the release of hypoxia inducible factor 1 alpha (HIF-1α), which is known to promote tumor progression and metastasis by inducing angiogenesis, immunosuppression, inflammation, resistance to cell death by apoptosis and autophagy, altered metabolism. Hypoxia ultimately causes drug resistance because various treatments, including radiation, chemotherapy, and even immunotherapies, require oxygen to be effective [41]. Many studies

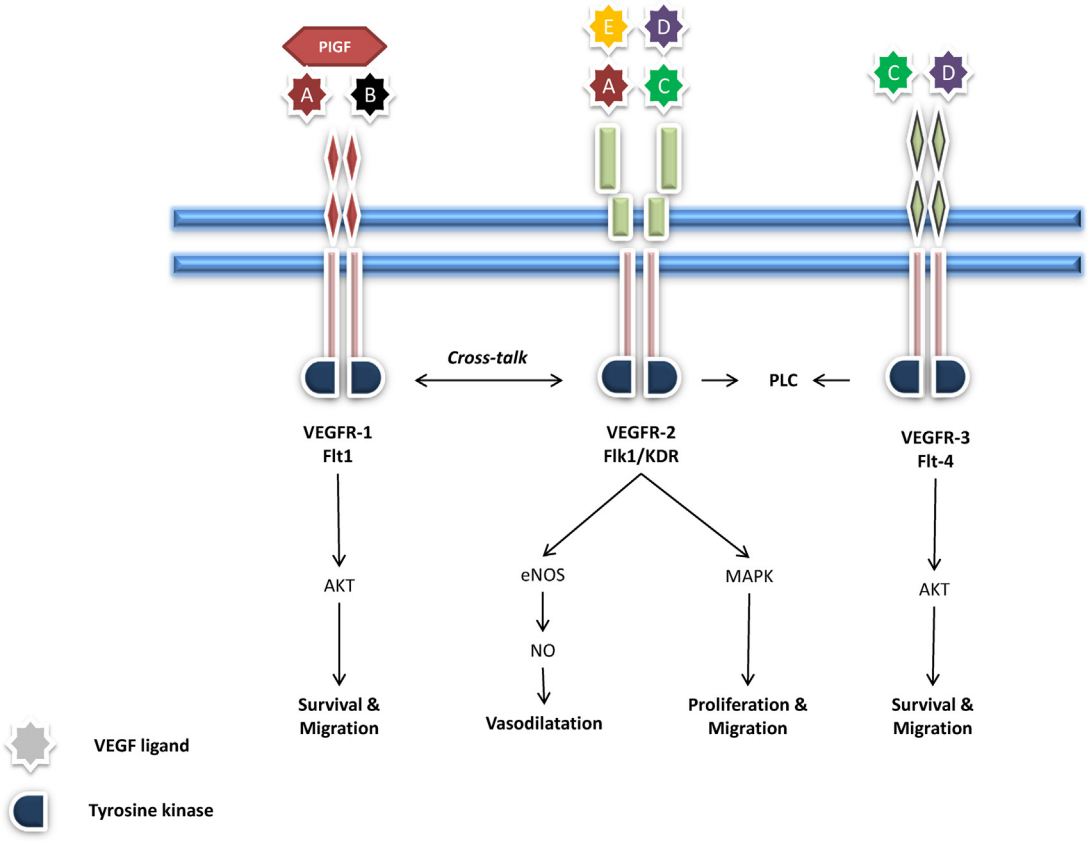


Fig. 1. Angiogenesis signaling pathway: receptors and ligands (A, VEGF-A; B, VEGF-B; C, VEGF-C; D, VEGF-D; E: VEGF-E; eNOS, endothelial nitric oxide synthase; MAPK, mitogen-activated protein kinase; NO, nitric oxide; PIGF, placental growth factor; PLC, phospholipase C; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor).

showed that in gastric cancer, HIF-1α activation correlates with an aggressive tumor phenotype and poor prognosis and it can occur also via hypoxia-independent mechanisms such as PI3K/AKT/mTOR signaling and reactive oxygen species (ROS) production. Published data demonstrate that suppression of HIF-1α decreases VEGF secretion in gastric cancer cells and impairs tumor growth and angiogenesis, showing that HIF-1α also contributes to the formation of a complex proangiogenic microenvironment in gastric tumors, thereby affecting the vessel morphology and function [42].

3. Pharmacogenomics of antiangiogenic inhibitors

Despite the large number of preclinical and clinical studies that tried to identify a predictive marker, at present no validated biomarkers are available for selecting candidates to antiangiogenic therapy or for monitoring their response during treatment. However, several surrogate biomarkers of response to antiangiogenic therapy have been described. Data obtained from in vitro and in vivo models of gastric cancer excluded the correlation between expression levels of angiogenic markers, phosphorylation of VEGFR, tumor microvessel density and polymorphisms of the VEGF gene whereas they showed an association between antiangiogenic treatment resistance and high levels of basic fibroblastic growth factor (bFGF), or between the VEGF/bFGF ratio that correlates more closely with sensitivity to antiangiogenic treatment, in particular bevacizumab [43]. Other published data identified VEGF tumor expression as a prognostic marker, showing that prognosis was significantly worse in patients who had high microvessel count (MVC) (16 or greater) or a high proliferating cell nuclear antigen (PCNA) (42% or greater) compared to patients whose tumor had a low MVC (less than 16) or a low PCNA (less than 42%); based on these results, tumor angiogenesis and tumor cell proliferation could be used as a prognostic indicators in gastric carcinoma [44]. In a study conducted on 91 radically resected gastric adenocarcinomas, the relationship between VEGF-D, VEGF-C, and VEGFR-3 immunoistochemical expression or mRNA levels and the clinical outcome was studied. The Authors reported a VEGF-D and -C expression in 67% and 50.5% of gastric cancers, respectively, while healthy gastric mucosa was negative for VEGF-C and positive for VEGF-D in 12.5%. According to the analysis conducted, higher levels of VEGF-C correlated with the risk for lymphatic spread and decreased survival. Moreover, the presence of abundant VEGF-C/VEGF-D/VEGFR-3 expression was

associated with poor outcome, allowing the identification of an unfavorable group of patients, and it could be considered as a potential negative prognostic factor [45]. The immunohistochemical analysis of the expression of VEGF-A and VEGF-C in 51 specimens of gastric carcinoma demonstrated that also VEGF-C expression in tumor tissues was indicative of lymphatic metastasis, whereas VEGF-A expression was more likely to be associated with hematogenous metastasis [46].

Because of the discordant published results, many groups focused their attention on the analysis of VEGF gene polymorphisms to help identifying patient subgroups at risk of poor outcome, but again results were inconsistent. Four-hundred-twenty-four patients with metastatic colorectal cancer treated with first line chemotherapy with FOLFIRI plus bevacizumab were recruited in order to test germline polymorphisms of: VEGFA g.43672669A > G, c.−2055A > C, VEGFR1 c.3635 + 319G > T and c.3639C > T, VEGFR2 c.359–1174G > A, c.2266 + 1166A > G and c.889G > A and EPAS1 c.780–1603G > A. No differences were observed in PFS or OS for all polymorphisms with the exception of VEGFR2 c.2266 + 1166 G variant, which was associated to shorter PFS and OS, with a trend toward statistical significance as compared to the AA genotype [47]. Five hundred and three patients with surgically-resected gastric adenocarcinoma were enrolled to test tissue VEGF polymorphisms c.2460T > C, c.2116G > A, c.+405G > C and c.+936C > T. The survival analysis showed no association of c.2460T > C, c.2116G > A, c.+405G > C VEGF gene polymorphisms with prognosis, but the c.+936TT genotype seemed to correlate with a worse OS compared with the CC genotype. In the haplotype analysis, the CACC haplotype was associated with a significantly worse survival when compared with the TGGC haplotype [48]. Genotyping for VEGF-A, VEGF-C, VEGFR-1,2,3 and integrin alpha −3 (ITGA) and −V (ITGV) was carried out in 101 patients with radically resected gastric tumors. At multivariate analysis, intestinal histology and the AC genotype of VEGFA c.−2055A > C were independently correlated with hematogenous metastases, whereas diffuse histology and the AA genotype of c.996C > T (ITGA) were independently correlated with peritoneal-only diffusion [49]. In conclusion, these results not only seem to indicate that combining information from expression and genotyping analysis and tumor histology could help clinicians to identify responders vs non-responders to antiangiogenic treatment, but they also underline the complexity of angiogenesis. Nevertheless, such approach is still far from being introduced in clinical practice.

4. Targeting VEGF: the example of bevacizumab

Bevacizumab, a recombinant humanized monoclonal antibody to VEGF-A, was studied for activity and safety for advanced GC treatment. Two different phase II clinical trials evaluated the addition of bevacizumab to cisplatin-based regimens [50,51]. Both trials reported a similar response rate (RR) of 67% but a different median overall survival (OS) which was of 12.3 months for the combination bevacizumab plus cisplatin and irinotecan [50] and 16.2 months for the combination of bevacizumab plus weekly docetaxel, 5-fluorouracil and cisplatin [51]. The activity of bevacizumab was evaluated also in combination with a different triplet regimen, including docetaxel, cisplatin and irinotecan [52] or oxaliplatin-based regimens [53,54]. Another prospective multicenter phase II study was designed to assess the activity and safety of capecitabine, oxaliplatin and bevacizumab in previously untreated advanced esophagogastric cancer [55]. Enrolled patients received capecitabine (850 mg/sqm twice daily on days 1–14) and oxaliplatin (130 mg/sqm) with bevacizumab (15 mg/kg on day 1 of a 21-day cycle); results showed a median PFS of 7.2 months, a median OS of 10.8 months and a RR of 51.4%. Based on the promising results of these pivotal phase II studies, 774 patients with advanced GC were enrolled in the large phase III AVAGAST trial and randomized to 5-fluorouracil/capecitabine and cisplatin with either bevacizumab (7.5 mg/kg iv) or placebo as first-line treatment [56]. In the global trial, enrolled patients were Asian (49%), European (32%) or American (19%). Patients without disease progression after six cycles were continued on 5-fluorouracil/capecitabine and bevacizumab until disease progression or unacceptable toxicity. Although the addition of bevacizumab to chemotherapy resulted in higher RR (46% vs 37.4%, $p = 0.0315$) and significantly prolonged median PFS (6.7 months vs 5.3 months, HR = 0.80, 95% CI 0.68–0.93, $p = 0.0037$), the study failed to meet its primary endpoint and no significant differences in OS were reported in the whole ITT population (median OS 12.1 months vs 10.1 months, HR 0.87, 95% CI 0.73–1.03, $p = 0.1$). Overall, AVAGAST trial suggested that the addition of bevacizumab to chemotherapy did not substantially increase the chemotherapy-related toxicity profile, with the possible exceptions of diarrhea (8% vs 4%) and HFS (6% vs 3%). Moreover, no new alert reporting safety concerns was recorded. Specific bevacizumab-related adverse events were similar between treatment arms, excluding hypertension (6% vs 1%) that, as expected, was more frequently reported in patients exposed to bevacizumab. Venous and arterial thrombotic events were less frequent in the experimental arm (7% vs 11%), and no differences in frequency or severity were reported for hemorrhage, proteinuria, and intestinal perforations or wound healing complications. In this study, notable differences across geographical regions were described. While the median survival benefit of adding bevacizumab appeared to be greater in American (+4.7 months, HR for OS 0.63) and European patients (+1.5 months, HR for OS 0.85), compared to Asian subjects (+1.8 months, HR for OS 0.97), Asian patients had the longest median survivals (12.1 months in the standard arm; 13.9 months in the experimental arm) possibly because of a higher use of second-line treatments. Other factors that may explain these survival data include ethnical differences in the proportions of patients with GEJ cancer, different tumor histology,

and genetic differences. The potential role of angiogenic biomarkers in predicting the response to bevacizumab efficacy in GC patients was also evaluated across different histological subtypes (proximal, non-diffuse; diffuse and distal non-diffuse). Translational research [57] suggested that a higher VEGF expression and lower neuropilin-1 (NRP-1) levels might be related with outcome. In the overall analysis, trend toward improved OS was reported for patients with higher baseline plasma VEGF-A (HR 0.72, 95% CI 0.57–0.93, interaction $p = 0.07$) or low basal expression of NRP-1 (HR 0.75, 95% CI 0.59–0.97, interaction $p = 0.06$). Both biomarkers, however, had statistically significant predictive value in the non-Asian cohort. In conclusion, plasma VEGFA and NRP-1 may help predicting the clinical outcome in patients with advanced gastric cancer treated with bevacizumab. Since proximal tumors have higher levels of NRP-1 and lower levels of plasmatic VEGF-A, non-Asian patients with diffuse and distal non-diffuse type tumors appeared to benefit the most from bevacizumab (median OS of 11.4 months with bevacizumab vs 7.3 months with placebo). Accordingly, in a smaller phase II trial, NRP-2 mRNA level significantly correlated with improved PFS ($p = 0.042$) and showed a trend toward longer OS ($p = 0.051$) [55]. Whether the different ethnic profile of genes involved in angiogenesis may impact on survival outcomes, was the primary aim of a recent pharmacogenomic study that specifically investigated the role of seven genetic polymorphisms in predicting the effect of bevacizumab [58], showing that Caucasians may be genetically favored to have antiangiogenic effect. The development of bevacizumab-induced hypertension during treatment was suggested to be associated with favorable clinical outcomes in metastatic colorectal cancer patients [59–61] although this issue is still debated [62]. However, the occurrence of hypertension in GC has not been reported to predict benefit from bevacizumab [63]. Recently, the AVATAR study has investigated the role of bevacizumab in combination with standard capecitabine and cisplatin in 202 Chinese patients with advanced GC [64]. The study did not meet its primary survival endpoint (HR for OS was 1.1), showing no benefit from the addition of antiangiogenic treatment to standard chemotherapy in this population. Despite the large-scale AVAGAST trial showing potential benefit of the antiangiogenic strategy in specific populations, the overall use of bevacizumab in patients with advanced GC conveyed unsatisfactory results and the drug was not licensed for clinical use. Blocking angiogenesis was indeed effective and beneficial for some GC patients as it is for patients with advanced colorectal or ovarian cancers. However, the overall effect may have been diluted when considering the whole population or when applying RECIST criteria that may not fully capture the activity of the drug.

Achieving a higher rate of RR is crucial especially for patients presenting with locally advanced disease who may still be suitable for salvage surgery. As a matter of fact, an active preoperative chemotherapy may potentially increase the chance for radical resection and this may translate into long-term survival benefit. Perioperative chemotherapy is a universally accepted option in this setting, based on the parallel results of two key randomized studies. Indeed, MAGIC [3] and the FNCLCC-FFCD cooperative trial [4] have both contributed to define perioperative chemotherapy as the standard of care in Europe for locally advanced GC patients, showing a convincing 13% absolute benefit increase in overall survival at 5 years for patients who undergo the perioperative strategy in the first trial and 14% in the second. The addition of bevacizumab and erlotinib to preoperative chemoradiation was recently assessed in a phase II study [65]. Sixty-two patients with localized esophageal or GEJ cancer received neoadjuvant chemoradiation, paclitaxel, carboplatin, and 5-fluorouracil with radiation therapy consisting of 1.8-Gy single fractions (with a total of 45 Gy) combined with bevacizumab (15 mg/kg IV) and erlotinib (100 mg orally). The study, however, failed to reach its primary endpoint of pathologic complete response (pCR) rate. The addition of bevacizumab to chemotherapy in this setting is currently being assessed in the phase II/III UKST03 (MAGIC-B) study [66]. This trial is recruiting 1100 resectable esophago-gastric cancer patients who will be randomized to perioperative chemotherapy with or without bevacizumab. Maintenance bevacizumab is given for a further 18 weeks after the completion of post-operative chemotherapy. The results of this study could clarify the role of bevacizumab in adjuvant/neoadjuvant setting.

Based on the results reported in the AVAGAST, the phase II NCT01471470 study has been designed to investigate the activity of docetaxel, cisplatin and capecitabine plus bevacizumab as induction chemotherapy in fit patients with upfront unresectable HER2 negative locally advanced GC. The primary endpoint of the study is R0 resection rate; secondary endpoints are OS, PFS, angiogenetic biomarkers and toxicity.

5. Targeting VEGFR-2: the example of ramucirumab

Among many novel antiangiogenic drugs, ramucirumab, a fully human IgG_1 monoclonal antibody specifically directed against VEGFR-2, is emerging as a new therapeutic option [67]. The phase III, placebo-controlled, double-blinded REGARD trial was conducted with the aim of evaluating the safety and efficacy of ramucirumab as second-line treatment in 355 patients with advanced, unresectable gastric or GEJ cancers. Patients were randomly assigned 2:1 to receive ramucirumab plus best supportive care or placebo plus best supportive care until disease progression, unacceptable toxicities or death. The primary endpoint of the trial was OS. At the end of the enrolment, the survival analysis was based on 278 events (179 patients in the ramucirumab arm and 99 in the placebo group). Treatment with ramucirumab resulted in a statistically significant OS improvement (HR 0.77, 95% CI 0.60–0.99, $p = 0.047$), with a median OS of 5.2 months for patients who received ramucirumab vs

3.8 months for those who received placebo. Moreover, an increase in PFS was reported (2.1 months for ramucirumab vs 1.3 months for placebo; HR = 0.48, $p < 0.0001$). This new antiangiogenic drug was very well tolerated, with a similar rate of grade 3 or grade 4 adverse events across study arms (56.8% in the ramucirumab vs 58.3% in the placebo group). Ramucirumab was not associated with higher toxicity rates, except for arterial thromboembolic events, which were slightly more common (2% vs 0%). The most frequent grade ≥ 3 adverse-events were: hypertension (8% in the ramucirumab group vs 3% in the placebo group), anemia (6% vs 8%), abdominal pain (6% vs 3%), fatigue (6% vs 10%) and anorexia (3% vs 3%) [68]. RAINBOW, a second international, randomized phase III trial, investigated the role of ramucirumab combined with paclitaxel in 655 advanced GC patients who had failed a first-line treatment comprising platinum and fluoropyrimidine [69]. The trial compared ramucirumab plus paclitaxel to placebo plus paclitaxel as second-line treatment in patients with metastatic gastric or junctional adenocarcinomas, with the results being recently presented [70]. In this study, the proportion of Asian patients was greater than the one included in the REGARD trial. The combination of ramucirumab and paclitaxel resulted superior in median OS (9.6 months vs 7.4 months; HR 0.81, 95% CI 0.68–0.96, $p = 0.017$), median PFS (4.4 months vs 2.8 months; HR 0.63, 95% CI 0.53–0.75, $p < 0.0001$), and RR (28% vs 16%, $p < 0.0001$) compared to standard treatment. Preplanned Forrest plot analyses confirmed the survival advantages in all subgroups. Interestingly, approximately 70% of Asian patients and almost 35% of European and American patients, received a post-discontinuation treatment. Severe grade of hypertension (14.1% vs 2.4%), fatigue (7% vs 4%), and neutropenia (40.7% vs 18.8%) were more frequently reported in patients exposed to ramucirumab, but the incidence of febrile neutropenia (3.1% vs 2.4%) was comparable between arms. Besides, the median duration of treatment was 18 weeks for the ramucirumab plus paclitaxel group and 12 weeks for the placebo group: such longer exposure to paclitaxel may account for the higher rate reported of any-grade peripheral neuropathy (14.4% vs 9%) in the experimental arm. As expected, the rate of patients that had received HER-2 inhibitors was low in both treatment arms (9% vs 8%). Interestingly, both the REGARD and the RAINBOW studies showed improbe quality of life results and longer time to clinical deterioration when the enrolled patients were exposed to ramucirumab. Based on the results of both trials and due to its specific mechanism of action, ramucirumab received its first global approval for use as monotherapy or in combination with paclitaxel in patients who experienced disease progression on or after fluoropyrimidine- or platinum-containing chemotherapy. A large phase II study is currently comparing first-line modified FOLFOX6 plus ramucirumab to modified FOLFOX6 plus placebo in the advanced disease setting. PFS is the primary trial endpoint with an estimated enrollment of 166 patients and the study accrual has been recently completed [71].

Available data from phase III trials of anti-angiogenic drugs in advanced GC are summarized in Table 1. Of note, the use of ramucirumabin combination with standard chemotherapy is currently being tested in a first-line phase III trial [72].

6. Blocking angiogenesis with oral TKIs and other angiogenic-inhibitors

Multitarget TKIs represent another potential approach to block angiogenesis by simultaneously targeting VEGFR and other signaling pathways. Oral TKIs with antiangiogenic properties, such as sorafenib or sunitinib, have already being tested in breast, lung or pancreatic cancer but with limited results, especially when used in combination with systemic chemotherapy. The most interesting and promising anti-angiogenic compounds are summarized in Fig. 2.

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 (PDGFR), VEGFR, KIT, Flt-3 and RET that impair tumor proliferation and angiogenesis [73]. 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 gastric or GEJ 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 [74]. 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 overall RR was limited to 3.9%, median PFS was 1.28 months (95% CI, 1.18–1.90), and median OS was 5.81 months (95% CI, 3.48–12.32). Serious adverse events occurred in 26 patients, leading to 13 treatment-related deaths [75]. Due to the lack of activity of sunitinib as single-agent in second-line treatment for advanced gastric cancer, its role has been therefore assessed also in combination with chemotherapy. In 2012, a Korean trial randomized 107 patients with unresectable or metastatic gastric cancer to single-agent docetaxel (60 mg/sqm, every 3 weeks) or docetaxel (60 mg/sqm every 3 weeks) in combination with sunitinib (37.5 mg/day). The primary endpoint of the study was TTP and the secondary endpoints included overall RR, disease control rate, OS and toxicity. The TTP was not significantly prolonged in the combination arm when compared with chemotherapy alone: 3.9 months (95% CI 2.9–4.9) vs 2.6 months (95% CI 1.8–3.5), with an HR of 0.77 (95% CI 0.52–1.16, $p = 0.21$). Although the objective RR was significantly higher in the docetaxel plus sunitinib arm (41.1% vs 14.3%, $p = 0.002$), patients exposed

Table 1
Antiangiogenic agents in advanced gastric cancer: phase III trials (BSC: best supportive care; CT: chemotherapy; HR: hazard ratio; ORR: overall response rate; OS: overall survival; PFS: progression free survival; NS: not significant).

Trials	Line	Treatments	ORR (%)	HR	PFS	HR	OS	HR OS
AVAGAST [55]	1°	CT/Placebo	37.4	($p = 0.0315$)	5.3	0.80 ($p = 0.0037$)	10.1	0.87 ($p = 0.1002$)
		CT/Bevacizumab	46		6.7		12.1	NS
AVATAR [63]	1°	CT/Placebo	29	($p = 0.3480$)	6	0.89 ($p = 0.4709$)	10.5	1.11 ($p = 0.5567$)
		CT/Bevacizumab	33	NS	6.3	NS	11.4	NS
REGARD [67]	2°	BSC/Placebo	3	0.76	1.3	0.48 ($p < 0.0001$)	3.8	0.77 ($p = 0.047$)
		BSC/Ramucirumab	3		2.1		5.2	
RAINBOW [69]	2°	CT/Placebo	16	($p < 0.001$)	2.8	0.63	7.4	0.81
		CT/Ramucirumab	28		4.4		9.6	

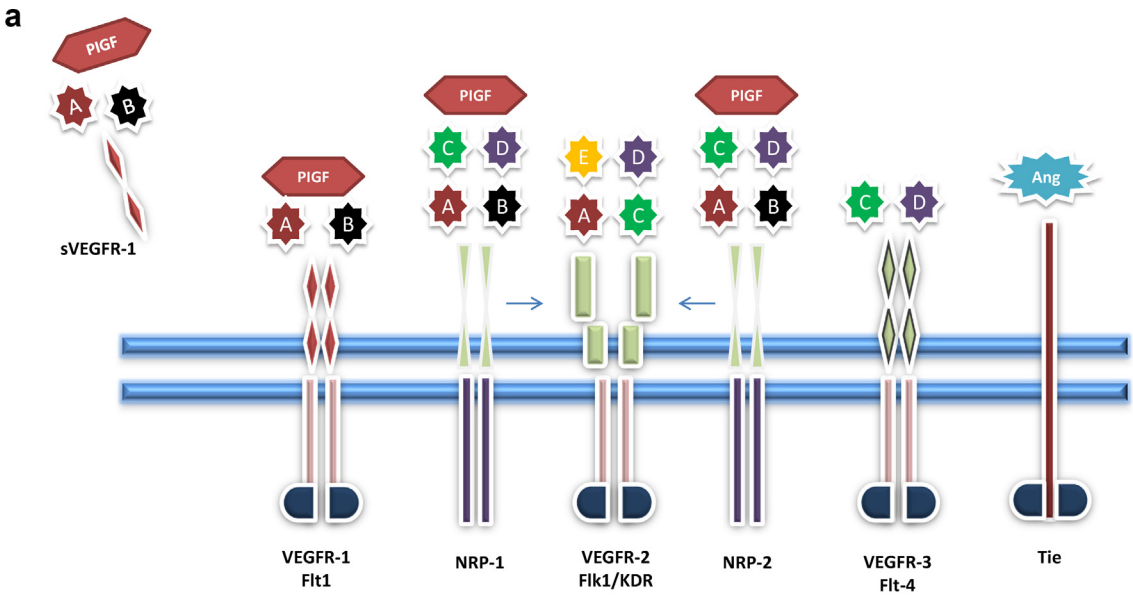
to the combination experienced more stomatitis, diarrhea, and hand–foot syndrome [76]. A recent phase I study evaluated the maximum tolerated dose (MTD), safety, pharmacokinetics and antitumor activity of sunitinib plus S-1 and cisplatin in patients with metastatic GC. The oral angiogenic inhibitor was 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/sqm). MTD of sunitinib was 25 mg/day; this regimen had a manageable safety profile and interesting antitumor activity. The most frequently reported severe or life-threatening adverse events were neutropenia (93.8%) and leucopenia (75.0%). The objective RR was 37.5%; six additional patients did not have any disease progression for ≥24 weeks. Median PFS was 12.5 months. No pharmacokinetic drug–drug interactions were observed between sunitinib and S-1 or cisplatin [77]. The combination of the intermittent schedule sunitinib with capecitabine and either cisplatin or oxaliplatin was equally safe and active [78].

Sorafenib is another TKI that may interfere 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/sqm bid, days 1–21) and CDDP (60 mg/sqm, 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 8 had stable disease as best response [79]. In another dose-finding study of sorafenib in combination with capecitabine and cisplatin as first-line treatment in patients with advanced GC sorafenib 400 mg bid daily, capecitabine800 mg/sqm bid (days 1–14), and cisplatin 60 mg/sqm (day 1) were the recommended phase II doses found. An objective RR 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 also reported [80]. A phase II study was subsequently conducted to determine the activity and toxicity of the three-weekly combination of sorafenib (400 mg bid continuously), docetaxel (75 mg/sqm day 1), and cisplatin (75 mg/sqm on day 1) in 44 patients with advanced disease. Partial response, the primary endpoint, was reported in 41% (18 patients) of the cases and 32% (14 patients) achieved stable disease. A median PFS of 5.8 months and a median OS of 13.6 months were also reported [81].

Cediranib (AZD2171) is a selective, highly potent oral molecule that competes with ATP for the binding to the intracellular domain of VEGF receptors [82]. In combination with cisplatin and S-1 or capecitabine, cediranib was tested in 14 previously untreated patients. The most common adverse events were decreased appetite, fatigue, and nausea (92.9%). Preliminary activity evaluation showed one confirmed and three unconfirmed PR [83]. Interestingly, this oral antiangiogenic compound seems to have activity in patients with pleural effusion or abdominal ascites [84] that frequently occur in advanced gastric patients.

Another new antiangiogenic compound is trebananib (AMG 386), an intravenously administered peptide-Fc fusion protein that neutralizes the interaction between angiopoietins-1 and -2 and the Tie2 receptor [85]. Trebananib is being studied in gastrointestinal [86], gynecological [87], and renal cancers [88]. In a phase II randomized, double-blind, placebo-controlled trial, 171 GC patients were randomized to cisplatin 80 mg/sqm plus capecitabine 1000 mg/m^2 bid every 3 weeks plus weekly AMG 386 at a dose of 10 mg/kg (arm A) or 3 mg/kg (arm B), or placebo (arm C). Median PFS, the primary study endpoint, was similar across treatment arms (4.2 months vs 4.9 months vs 5.2 months, respectively; HR 0.98 for arms A + B vs Arm C; 95% CI 0.67–1.43; $p = 0.92$). Accordingly, objective RR was also similar. Incidence of grade ≥3 adverse events was 80% in arm A, 84% in arm B, and 75% in arm C. The most frequently reported severe adverse events were fatigue, abdominal pain, and gastrointestinal toxicities [89].

Apatinib (YN968D1) is a novel, highly potent VEGFR-2 inhibitor with a binding affinity 10 times that of sorafenib [90]. Based on the results of a previous phase I trial showing some anticancer activity in Chinese patients with metastatic GC, a phase II randomized, double blind, placebo-controlled trial was conducted to test this new drug in heavily pretreated GC patients. The aims of this study were 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 (group A), apatinib



b

BEVACIZUMAB	RAMUCIRUMAB	APATINIB FORETINIB MGCD265 REGORAFENIB SUNITINIB SORAFENIB TSU-68	CEDIRANIB	TREBANANIB
Human monoclonal antibody	Human monoclonal antibody	Multitarget tyrosine kinase inhibitors	VEGF tyrosine kinase inhibitor	Peptibody
A	VEGFR-2 Flk1/KDR			Ang

Fig. 2. (a) Angiogenesis signaling pathway and promising molecular-targeted therapy for advanced gastric cancer (A, VEGF-A; Ang, angiopoietin; B, VEGF-B; C, VEGF-C; D, VEGF-D; E, VEGF-E; NRP, neuropilin; PIGF, placental growth factor; sVEGFR, soluble vascular endothelial growth factor receptor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor). (b) Promising new drugs for advanced gastric cancer and corresponding targets (A, vascular endothelial growth factor A; Ang, angiopoietin VEGFR, vascular endothelial growth factor receptor).

850 mg once daily (group B), or apatinib 425 mg twice daily (group 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 9 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 [91].

Apatinib appears to have a favorable safety profile because it is a relatively selective tyrosine-kinase inhibitor. In view of the better toxicity profile, the once-daily schedule was chosen to be further investigated and is currently being tested in an ongoing randomized phase 3 trial [92].

Regorafenib, an oral multitarget TKI [93], which has already been approved as salvage treatment for highly pretreated metastatic colorectal cancer and GIST patients, appeared to be active in preclinical studies also in gastric cancer showing significant tumor growth inhibition, but phase II and III trials are necessary to further investigate its potential therapeutic role [94].

R1498 is an orally active kinase inhibitor that targets both the angiogenic and the mitotic pathways. Specifically, this novel compound may potently inhibit Aurora A kinase, a potent tumor growth promoter in preclinical GC models [95]. In vivo, anti-tumor efficacy of R1498 was evaluated on a panel of GC and HCC xenografts in a parallel comparison with another multikinase inhibitor sorafenib. R1498 demonstrated superior efficacy and better toxicity profile over sorafenib in all tested models with high tumor growth inhibition and tumor regression. The therapeutic potential of R1498 was also highlighted by its efficacy on three human GC primary tumor derived xenograft models with 10–30% tumor regression rate [96].

Foretinib (GSK136089) is an oral multikinase inhibitor with antiangiogenic properties, known to target MET, RON, AXL, and VEGFRs [97]. Moreover, the compound may potently inhibit ROS1 rearrangements [98]. Originally studied in papillary renal carcinomas [99], foretinib appears active against GC cells harboring not only MET but also FGFR2 amplification, suggesting an inhibitory effect by blocking inter-RTK signaling networks with MET or FGFR2 at their core [100]. A multicenter, phase II study tested two different schedules of oral foretinib: the intermittent schedule (240 mg/day for 5 consecutive days every 2 weeks) and the daily dosing (80 mg/day during each 2-week cycle). Overall, 74 patients were enrolled, 93% of them had received prior therapy. Stable disease was reported in 10 patients (23%) receiving the intermittent schedule and in 5 subjects (20%) receiving the daily dosing. Tumor samples were available for molecular analysis for 67 patients: among them, 3 had *MET* amplification, one of which achieved SD. Treatment-related adverse events occurred in 91% of patients. Rates of hypertension (35% vs 15%) and elevated aspartate aminotransferase (23% vs 8%) were higher with the intermittent dosing. Despite the strong preclinical evidence of MET inhibition, single-agent foretinib did not show any efficacy in unselected patients with metastatic GC [101]. It should be noted that the role of MET as novel potential target has been recently challenged by the disappointing results of two different trials investigating onartuzumab and rilotumumab respectively, in this disease setting [102,103].

MGCD265 is a potent inhibitor of the receptor tyrosine kinases (RTKs) MET, VEGFR 1, 2, 3 and Axl. Early clinical trials are ongoing and assessing the role of MGCD265 as single agent or in combination with antineoplastic agents. In vitro experiments have demonstrated that MGCD265 may inhibit cancer cell motility and proliferation and MET-mediated signal transduction with nanomolar potency. Also, the compound may induce apoptosis in cancer cells and blocks the activation of MET mutant clones. MGCD265 has demonstrated a favorable safety profile; moderate drug-related diarrhea, fatigue and lipase elevation were observed in patients exposed to the drug, while the maximum tolerated dose has not been defined yet. Long-term survival was reported in NSCLC ($n=2$), GEJ cancer ($n=2$), ovarian cancer ($n=1$), and chordoma ($n=1$) patients treated with combination therapy. Disease control was obtained in 11 out of 12 NSCLC patients (2 partial responses) in combination with docetaxel and in 4 out of 9 GE/GEJ cancer patients (2 non-CR/non-PD) in combination with erlotinib [104].

TSU-68 (Orantinib) is a novel oral agent that inhibits tyrosine kinase phosphorylation of VEGFR-2, PDGFR, and FGFR-1. The antitumor activity of TSU-68 was initially evaluated in Asiatic patients [105–109]. A randomized phase II study enrolled advanced GC patients in order to evaluate the activity of TSU-68 in combination with S1 and cisplatin. No difference in PFS was observed between the two arms of chemotherapy plus TSU-68 or chemotherapy alone (6.8 months vs 7 months, $p=0.424$). Accordingly, OS ($p=0.213$) and objective RR ($p=0.671$) were also similar. Overall, liver enzyme alteration, vomiting, diarrhea, abdominal pain, anorexia and edema occurred more frequently in patients treated with TSU-68. No differences in grade 3 or higher adverse events were observed between treatment groups, with the exception of anorexia and hematological toxicity. Other studies are required to explore the potential role of this molecule in gastric cancer [110].

Pazopanib is another novel well-known molecule [111]. A phase II trial is currently ongoing to evaluate the safety and efficacy of pazopanib in combination with an oxaliplatin-based therapy as first-line combination for patients with advanced gastric cancer [112]. Ombrabulin is a novel vascular disrupting agent being investigated in phase I trials including gastrointestinal cancers [113,114].

Available data from phase II trial of new angiogenic-inhibitors are summarized in Table 2. All these molecules may offer new therapeutic options in this disease. However it should be underlined the need for identifying predictive factors and major drivers of the disease. These efforts will allow physicians to select responding patients and are likely to maximize the benefit-to-risk ratio.

7. Future perspectives

While the benefit of palliative systemic chemotherapy has reached a plateau in the past two decades, the possibility of treating GC patients with novel drugs has recently emerged.

Table 2
Antiangiogenic agents in advanced gastric cancer: phase II trials.

Drugs	[73]	[79]	[87]			[89]			[99]		[106]	
	Sunitinib	Sorafenib +CT	Trebananib 10 mg/kg +CT	Trebananib 3 mg/kg + CT	Placebo +CT	Apatinib 850 mg once daily	Apatinib 425 mg twice daily	Placebo	Foretinib 240 mg/day d1-5,q14	Foretinib 80 mg/day	Orantinib +CT	CT
Line	2°	1°		1°			3° or later			2°		1°
ORR (%)	3.9	41	27	43	35	0	0	0	0	0	62.2	56.5
				p NS								*p* = 0.671
mPFS (months)	1.28	5.8	4.2	4.9	5.2	3.7	3.2	1.4	1.6	1.8	6.8	7
				p NS			*p* <0.001		*p* NR			*p* = 0.424
mOS (months)	5.81	13.6	NR	NR	NR	4.8	4.3	2.5	7.4	4.3	16.3	15.2
							p <0.001		*p* NR			*p* = 0.213

Among these drugs, antiangiogenic compounds seem to be of great importance. As expected, a more profound gain in the disease biology will lead to the discovery of novel driver pathways which may be further targeted with active drugs. In line with this, a massive effort has been made by The cancer genome atlas (TCGA) [115] in order to identify all the potential gene expression patterns that may justify tumor heterogeneity. Using six genomic and molecular platforms including DNA or RNA sequencing, protein arrays and sophisticated statistical and informatics analyses of data from 295 tumors, the TCGA network has proposed a novel GC classification based on four genomic subtypes. The role of novel antiangiogenic drugs may be of particular relevance in the CIN subtype, in which TCGA network identified genomic amplifications of many RTK. In this molecular subgroup recurrent amplification of *VEGFA* gene has been reported, and it will be interesting to investigate whether the response to ramucirumab may be predicted by the *VEGFA* amplification. The results of these analyses provide a new guide to targeted agents that should be formally tested in prospective clinical trials for distinct populations of GC patients. Nevertheless, disease anatomy and classical biology parameters are still important and should be integrated with novel molecular classifications.

Understanding and targeting the mechanisms of resistance to antiangiogenic drugs is another key point in order to improve the outcome for patients with advanced GC. Mechanisms of resi stance can be VEGF-axis dependent, stromal-dependent or associated with non-VEGF modulators [116]. Overcoming such mechanisms, will ensure a better use and results of most novel antiangiogenic drugs.

8. Conclusions

Although a pivotal randomized trial testing bevacizumab provided initial disappointing results, a growing amount of emerging data confirm that multiple mechanisms account for the efficacy of angiogenic inhibitors in patients with gastric cancer, and these data have renewed the interest for antiangiogenic therapy in this population. Ramucirumab, a novel VEGFR2 inhibitor, demonstrated efficacy in pretreated patients either as single-agent or in combination with weekly paclitaxel. Similarly, apatinib, another VEGFR2 inhibitor, showed promising results in a phase II trials, suggesting that VEGFR2 plays a key role in gastric cancer and that its inhibition may be associated with improved outcomes. Future efforts in translational research are aimed at clarifying which patients may specifically benefit from antiangiogenic treatments, which are the main mechanisms for intrinsic or acquired resistance, and how to better combine these molecules to maximize their effect in the clinical practice. Along this line, the development of other angiogenic inhibitors will enlarge the treatment landscape of this class in gastric cancers and gastroesophageal malignancies.

Conflict of interest

Giuseppe Aprile was involved and principal investigator in the REGARD Study and served as speaker for Roche, Merck-Serono, Eli-Lilly and Amgen.

Gianpiero Fasola participated in advisory boards for Amgen and served as speaker for Amgen, Eli-Lilly, Merck-Serono, Roche, Pfizer, and Glaxo.

All other authors have no significant conflicts of interest to disclose.

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Biography

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