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Review

REVIEW

Targeted therapy in gastroesophageal cancers: past, present and future

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Abstract

Gastroesophageal cancer is a significant global problem that frequently presents at an incurable stage and has very poor survival with standard chemotherapy approaches. This review will examine the epidemiology and molecular biology of gastroesophageal cancer and will focus on the key deregulated signaling pathways that have been targeted in the clinic. A comprehensive overview of clinical data highlighting successes and failures with targeted agents will be presented. Most notably, HER2-targeted therapy with the monoclonal antibody trastuzumab has proven beneficial in first-line therapy and has been incorporated into standard practice. Targeting the VEGF pathway has also proven beneficial, and the VEGFR-targeted monoclonal antibody ramucirumab is now approved for second-line therapy. In contrast to these positive results, agents targeting the EGFR and MET pathways have been evaluated extensively in gastroesophageal cancer but have repeatedly failed to show benefit. An increased understanding of the molecular predictors of response to targeted therapies is sorely needed. In the future, improved molecular pathology approaches should subdivide this heterogeneous disease entity to allow individualization of cancer therapy based on integrated and global identification of deregulated signaling pathways. Better patient selection, rational combinations of targeted therapies and incorporation of emerging immunotherapeutic approaches should further improve the treatment of this deadly disease.

Key words: esophageal cancer; gastric cancer; targeted therapy; molecular oncology

Introduction

The treatment of gastroesophageal cancers (GE-Cas) remains a significant clinical challenge. Intensive and toxic multimodality therapy for locoregional disease fails to cure the majority of patients, and standard chemotherapy for metastatic disease provides only short-term benefits. Our understanding of the molecular pathogenesis and biology of GE-Ca has increased significantly, leading to new and targeted therapeutic strategies that promise to increase patient survival while decreasing toxicity. The majority of these novel therapies target key signaling pathways that are deregulated in GE-Ca. In this review, we will briefly examine the epidemiology and molecular biology of GE-Ca. We will then discuss the major receptor tyrosine kinase signaling pathways commonly implicated in the pathogenesis of GE-Ca including EGFR, HER2, VEGF and MET. We will provide an update on trials targeting each of these in the clinic and highlight both the successes and the failures. While further advances are clearly needed, these strategies represent a new era in the treatment of GE-Ca and should continue to translate into longer survival outcomes for patients in the future.

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Epidemiology and molecular biology of gastroesophageal cancers

Esophageal cancer

The incidence of esophageal cancer has been rising in Western populations over the past few decades. It is estimated that 16 980 new cases of esophageal cancer will be diagnosed in the United States in the year 2015, with 15 590 deaths being expected [1]. Worldwide, an estimated 455 800 new esophageal cancer cases and 400 200 deaths occurred in 2012 [2,3]. There are two major histologic types of esophageal cancer: squamous cell carcinoma (SCC) and adenocarcinoma. Although SCC remains the most common histology worldwide, there has been a substantial shift in the histology of esophageal cancer in the Western countries. Since approximately the 1970s, the most common type of esophageal cancer in the United States has shifted from SCC to adenocarcinoma. Rates for SCC have constantly decreased, presumably due to long-term reductions in tobacco use and alcohol consumption (the two biggest risk factors for this histologic type) [4]. In contrast, the rates of adenocarcinoma of the esophagus have risen steadily, in part due to increases in known risk factors including obesity as well as Barrett's esophagus, gastroesophageal reflux disease (GERD), smoking, white race and male sex [5]. While these risk factors are also relevant to non-Western populations, the markedly increased incidence of predominantly SCC esophageal cancer worldwide suggests that additional unidentified risk factors also exist. With advances in treatment, patient survival has improved. Five-year survival was a dismal 5% in the mid 1970s compared with ~20% now. While this represents significant progress, survival still remains poor [1].

There are substantial genetic differences between esophageal adenocarcinoma and SCC [6]. Adenocarcinoma evolves from Barrett's esophagus, as evidenced by the fact that most driver mutations found in adenocarcinoma are already present in the preceding Barrett's esophagus lesions. On the other hand, esophageal squamous dysplasia precedes SCC [7]. On a genetic level, there is significant disparity in the mutation spectrum of the two cancer types. Notably, there are more insertions/deletions (indels) and C:G>G:C transversions in SCC, while A:T>C:G transversions are more common in adenocarcinoma. These mutations appear to impact distinct molecular pathways in SCC compared with adenocarcinoma. For example, inactivating mutations of *NOTCH1* were identified in 21% of SCCs but were not observed in adenocarcinoma [6]. These described pathologic and molecular differences are all too often ignored when designing clinical trials, which tend to pool SCC and adenocarcinoma together during both recruitment and analysis, and therapies benefiting specific histologic subtypes remain poorly defined.

Gastric cancer

Although it is relatively uncommon in Western populations, gastric cancer was the leading cause of cancer deaths globally until the 1980s [8] and remains one of the most common cancers worldwide [9]. It is estimated that 24 590 new cases of gastric cancer will be diagnosed in the United States in the year 2015, with 10 720 deaths expected [1]. In 2012, gastric cancer developed in approximately 930 000 individuals and accounted for 10% of cancer-related deaths [2]. The incidence and mortality of gastric cancer have declined since World War II [10], although the reasons for the decline are likely multifactorial and include the recognition of *H. pylori* and other environmental risks, the development of refrigerators and overall improvements in living standards. Studies of Japanese migrants to the United States suggest that early exposure to key environmental factors may have a greater influence than genetics on gastric cancer incidence and mortality [11]. The use of screening programs in Asia and advances in multimodality treatment have led to improved survival rates for patients with gastric cancer. Five-year relative survival rates for all stages are ~30%. However, only incremental progress has been made in the treatment of metastatic disease, in which the median survival remains poor at 8–10 months [12,13].

Histologically, gastric cancer is generally divided into intestinal and diffuse (infiltrative) subtypes. The intestinal-type gastric cancers are likely linked to environmental factors such as *H. pylori* infection and are more common in older age groups. The diffuse or infiltrative type, which is associated with Epstein-Barr virus (EBV) infection and hereditary mutations in *CDH1,* is more common in younger age groups and has a worse prognosis than the intestinal type [14]. The relative frequencies of each subtype are 54% intestinal, 14% diffuse and 32% mixed [15]. These issues become more complicated when considering tumor location as the tumors in the distal stomach are likely distinct from those found in the distal esophagus, gastroesophageal junction (GEJ) or proximal stomach. Given the limited utility of subtyping by histology or location, recent efforts have used comprehensive genetic analyses to define four molecular subtypes of gastric cancer: microsatellite unstable (21.6%), chromosomally unstable (49.8%), genomically stable (19.6%) and EBV related (8.8%) [16]. It is hoped that these molecular classifications will have more clinical relevance than the anatomical classifications described above. However, as in esophageal cancer, clinical trials have not focused on treatment by histology or molecular subtype, and these pathologic and genetic differences are currently of limited use when choosing therapies for patients.

While future trials will likely apply genetic classifications to both recruitment and analysis, the majority of clinical trials discussed in this review do not take molecular classifications into account. In fact, most have pooled analyses of patients with gastric, gastroesophageal and/or esophageal cancers, especially adenocarcinoma. This is justified, in part due to the considerable overlap of epidemiologic and molecular features of gastric and distal esophageal adenocarcinoma (although perhaps less justified for SCC). For this reason, the term GE-Ca will be used throughout this review.

Aberrant Receptor Tyrosine Kinase Pathways With Therapeutic Implications in Gastroesophageal Cancers

Many cell surface growth factor receptor pathways have been implicated in the pathogenesis of GE-Ca. An overview of these signaling pathways and the drugs that target them is presented in **Figure 1**. In the following sections, we will discuss the molecular biology and epidemiology of each pathway and will then review the experience with specific targeted agents in the clinic.

The EGFR Pathway

EGFR (epidermal growth factor receptor) is a receptor tyrosine kinase that is often overactive in human cancers and is thought to play a critical role in oncogenesis. It belongs to the ErbB family, which also includes ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4) [17]. EGFR remains in a state of auto-inhibition in the absence of ligands such as EGF and transforming growth

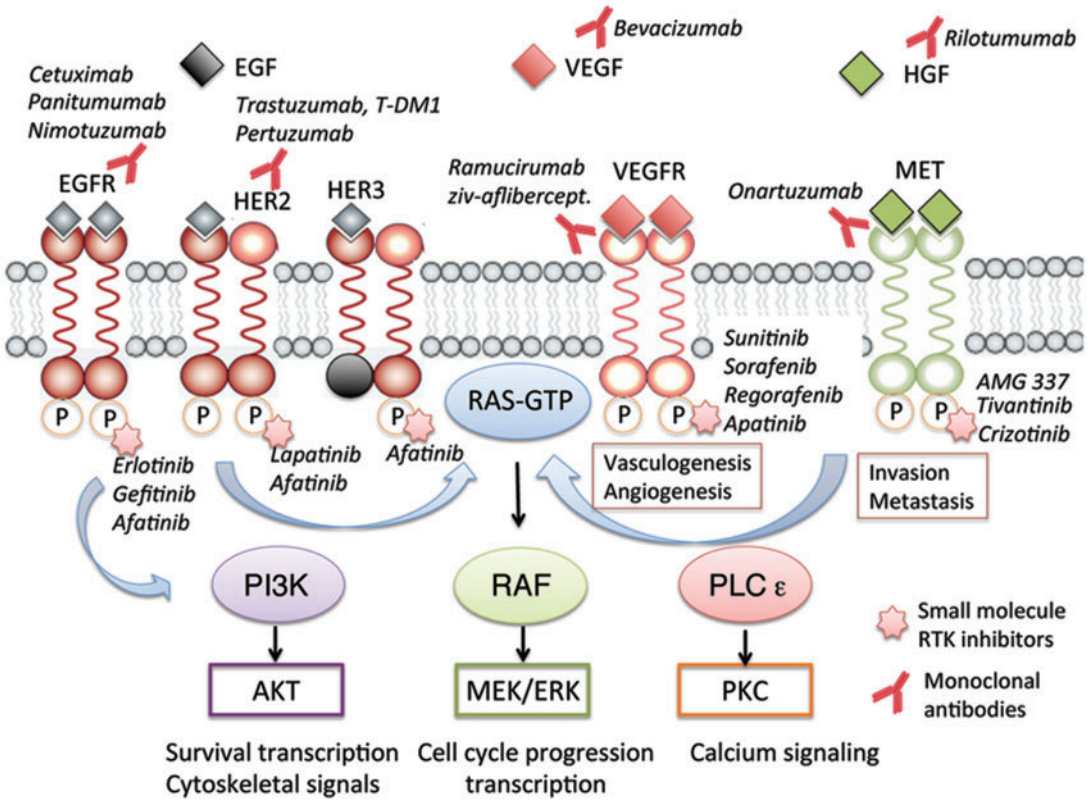

Figure 1. Receptor tyrosine kinases and their targeted therapies in gastroesophageal cancers: EGFR, VEGFR and MET. The EGFR family of receptor tyrosine kinases (RTKs) includes four distinct receptors: the EGFR (also known as ErbB-1/HER1), ErbB-2 (neu, HER2), ErbB-3 (HER3) and ErbB-4 (HER4). All proteins of this family have an extracellular ligand-binding domain, a single hydrophobic transmembrane domain and a cytoplasmic tyrosine kinase-containing domain. The intracellular tyrosine kinase domain of EGFR receptors is highly conserved, although HER3 lacks kinase activity. HER2 functions as a ligand-less receptor and induces hetero-dimerization with other EGFR family receptors upon ligand binding, whereas EGFR and HER4 undergo homo-dimerization. The subsequent activation of the intrinsic tyrosine kinase domain activates phosphorylation cascades, and the downstream effectors include RAF, PI3K and PLC. VEGF and its receptor VEGFR promote angiogenesis. The overexpression of VEGF is significantly associated with poor prognosis in gastrointestinal cancers. The HGF/MET pathway activates complex signaling events that depend on the cellular context and produces a variety of cellular responses such as proliferation, motility, angiogenesis and invasion. The MET pathway is upregulated in a wide range of human tumors, and this finding often signals a poor prognosis.

factor alpha (TGFα). After ligand binding, the receptors undergo homo- or hetero-dimerization with other members of the ErbB family. Dimerization triggers auto-phosphorylation of the EGFR intracellular domain leading to the subsequent activation of downstream signaling pathways [18]. Aberrant EGFR signaling leads to several hallmarks of cancer including increased proliferation, angiogenesis, metastasis and resistance to apoptosis [19].

EGFR is an attractive target in GE-Ca. In esophageal cancer, the overexpression of EGFR as assessed by immunohistochemistry (IHC) is reported in 40–80% of patients and occurs in both adenocarcinoma and SCC [20]. Numerous studies have shown that increased EGFR expression is associated with poor prognosis [21]. EGFR overexpression was observed in a smaller subset of patients with gastric cancer (27% by IHC) but was similarly associated with an unfavorable prognosis [22,23].

Clinical results of agents targeting the EGFR pathway

The EGFR pathway is an established target in colorectal cancer, SCC of the head and neck and lung cancer. EGFR is overexpressed in GE-Ca and is a rational target for therapy based on preclinical studies. Two classes of agents targeting the EGFR pathway are available for use in patients: monoclonal antibodies (mAbs), which recognize the extracellular domain of EGFR (cetuximab, panitumumab and nimotuzumab), and small molecule tyrosine kinase inhibitors (TKIs), which block intracellular signaling by EGFR (gefitinib and erlotinib). Here we will review the major clinical trials testing EGFR-targeted therapies in GE-Ca. Biomarkers relevant to other cancer types do not appear to be relevant in GE-Ca, and the majority of existing data are from unselected GE-Ca patient populations.

Anti-EGFR mAbs: cetuximab, panitumumab and nimotuzumab

Cetuximab and panitumumab target the extracellular domain of EGFR and are currently approved as therapy for multiple cancers including RAS-wildtype colorectal cancer in which they appear to be interchangeable in terms of efficacy [24]. Both of these agents have been tested extensively in metastatic GE-Ca as well, but their benefit is unclear at this time. Phase II trials of single-agent cetuximab have shown minimal activity [25,26]. However, multiple phase II studies of cetuximab in combination with chemotherapy have shown promise, with median overall survival (OS) of 9–11 months [27–32]. These encouraging findings led to two large phase III trials: EXPAND and REAL-3.

The EXPAND trial randomized 904 patients with metastatic GE-Ca to receive either placebo or cetuximab in addition to cisplatin/capecitabine chemotherapy [33]. The primary endpoint was progression-free survival (PFS), and secondary endpoints included overall survival (OS), response rate (RR) and toxicity. None of the endpoints differed significantly between placebo and cetuximab groups (PFS ~5 months, OS ~10 months, RR

30%), and trends unexpectedly suggested inferior survival with the addition of cetuximab. Toxicity was similar except for increased rash in the cetuximab group.

The REAL-3 trial randomized 503 patients to receive either placebo or panitumumab in addition to epirubicin/oxaliplatin/capecitabine (EOX) chemotherapy. Dosing of oxaliplatin and capecitabine were attenuated in the panitumumab arm due to the increased toxicity observed in a previous dose-finding study [34]. The primary endpoint was OS with secondary endpoints of PFS, RR and toxicity. Just as in EXPAND, the addition of targeted therapy led to inferior results and resulted in early termination of the trial. Patients in the panitumumab arm had an OS of 8.8 months, while those treated with placebo had an OS of 11.3 months. PFS (6.0–7.4 months) and RR (~40%) were similar between arms. Toxicity was increased with panitumumab, with increased incidence of grade 3–4 diarrhea and rash. As has been seen in previous trials, rash appeared predictive of response to treatment. Other biomarkers were examined including *RAS* mutational status. However, the numbers were too small to draw any definite conclusions [35,36].

A phase II randomized trial using an alternative chemotherapy regimen (docetaxel/oxaliplatin) with or without cetuximab as first-line therapy for unselected patients with metastatic GE-Ca (NCT00517829) showed similarly negative results, with a slight increase in RR in the cetuximab arm (27% in chemo alone *vs* 38% with cetuximab) but no difference in survival (PFS ~5 months, OS ~9 months) [37]. Notably, this trial was amended to allow *RAS* mutational testing on a subset of tumor samples. However, just as in REAL-3, no conclusions could be drawn regarding *RAS* as a biomarker in this clinical setting.

A third antibody targeting EGFR, nimotuzumab, has been extensively investigated in GE-Ca with promising results reported in single-arm studies. However, a randomized phase II study of nimotuzumab in combination with cisplatin/S-1 (an oral fluoropyrimidine) showed inferior outcomes for patients receiving this targeted therapy, and thus further studies have been halted [38]. Finally, many studies have incorporated anti-EGFR antibodies into chemoradiation regimens for localized disease. These studies have almost uniformly shown increased toxicity without clear increases in efficacy [39–43]. One prominent example was the phase II/III SCOPE1 trial that tested the addition of cetuximab to cisplatin/5-FU chemoradiation for definitive treatment of localized GE-Ca. Again, the cetuximab group experienced increased toxicity and had inferior outcomes, with an increased rate of disease progression at 24 weeks and a lower OS [39, 44]. While there are some ongoing clinical trials based on available data, there is currently no role for the anti-EGFR antibodies in the treatment of GE-Ca.

Anti-EGFR TKIs: gefitinib and erlotinib

The EGFR TKIs gefitinib and erlotinib have been tested in advanced GE-Ca, but their benefits have also not been proven yet. Multiple phase II trials suggested benefit [45–48]. This led to the randomized phase III COG trial (NCT01243398), which enrolled 450 patients with metastatic adenocarcinoma or SCC of the esophagus/GEJ who had disease progression after first-line chemotherapy. Patients were randomized to single-agent gefitinib 500 mg daily or placebo. A non-significant improvement in PFS (1.6 months gefitinib *vs* 1.2 months placebo) was observed. More importantly, the primary endpoint of OS did not differ between treatment groups (~3.7 months). Odynophagia was improved in the gefitinib group, but other symptoms were similar between groups.

While the overall results of the COG trial were negative, a small subgroup of patients had rapid disease response and durable disease control, suggesting that a not-yet identified biomarker could predict which patients were most likely to benefit from gefitinib therapy [49]. The TRANS COG analysis evaluated the predictive value of EGFR copy number gain (CNG) in 295 patients treated on the COG trial using prospectively collected tumor samples. CNG was evaluated using fluorescence in situ hybridization (FISH) on formalin-fixed samples [50]. Forty-six (15.6%) patients had evidence of CNG and appeared to benefit from gefitinib with improved OS, PFS and disease control rate (DCR). Notably, 38% of CNG patients treated with gefitinib survived 6 months, and 13% survived 12 months, which is comparable to survival in other second-line trials. The authors concluded that EGFR CNG may predict benefit from gefitinib and other EGFR-targeted therapies. While these results are provocative, they will need to be validated in additional studies before gefitinib could be considered for standard clinical practice.

EGF-targeted therapies: conclusions

In summary, despite adequate preclinical rationale, the results of EGFR-targeted therapies in GE-Ca have been disappointing, and these agents cannot be recommended at this time. It is interesting to note that, in most clinical situations in which EGFR inhibition has shown significant benefit, there are biomarkers available that predict response (e.g. *RAS* wild-type status in colorectal cancers and activating mutations in EGFR in lung cancer), but these do not appear to be relevant to GE-Ca. It is certainly possible that the identification of a reliable biomarker for response, such as EGFR CNG, would revitalize interest in these agents in the future.

The HER2 Pathway

HER2 (human epidermal growth factor receptor 2) is a second member of the ErbB family involved in GE-Ca [51]. HER2 can hetero-dimerize with any of the three other members of the ErbB family, which leads to the auto-phosphorylation of tyrosine residues in the cytoplasmic domain of the receptors and subsequent activation of downstream signaling pathways. This results in recruitment of transcription factors that modulate gene expression to drive cell-cycle progression, proliferation, survival and ultimately tumorigenesis [52]. The unprecedented success of HER2 targeting in breast cancer supports the critical importance of HER2 in the control of cancer growth and survival [53].

Approximately 30% of gastroesophageal adenocarcinomas overexpress HER2, which is comparable to the rates in breast cancer [54]. HER2 positivity rates were higher in GEJ cancer than in gastric cancer (33.2% *vs* 20.9%) and higher in intestinal cancer than in diffuse or mixed pathologic subtypes (32.2% *vs* 6.1%/20.4%) [55]. However, a greater degree of intratumoral HER2 heterogeneity is seen in GE-Ca compared with breast cancers [56]. Importantly, the criteria for HER2 overexpression in determining the eligibility of anti-HER2 therapy differ from those used in breast cancer [57]. GE-Cas that are IHC 3+ or FISH-positive are generally eligible for HER2-targeted therapies [58,59].

The association between HER2 expression and prognosis in GE-Ca remains controversial compared with the established adverse prognostic role of HER2 overexpression in breast cancer. For example, six trials of first-line chemotherapy that involved 381 patients with GE-Ca were retrospectively analyzed, and multivariate analysis indicated that HER2 status was not an

independent prognostic factor [60]. However, analysis of the control groups from two trials discussed later (ToGA and EXPAND) suggest that HER2-positive patients have a more favorable prognosis than HER2-negative patients, even when treated with chemotherapy alone [59,61].

Clinical Results of Agents Targeting the HER2 Pathway

HER-targeted agents are well established for the treatment of breast cancer, where four agents are currently approved: the mAbs trastuzumab, trastuzumab emtansine (T-DM1), pertuzumab; and the TKI lapatinib. Here, we will review mature results testing HER2-targeted therapies in GE-Ca but will also briefly review new strategies that are currently under investigation.

Anti-HER2 mAbs: trastuzumab, trastuzumab emtansine (T-DM1), and pertuzumab

Trastuzumab is a mAb that recognizes an extracellular epitope on the HER2 receptor. It has proven effective in the treatment of HER2-positive breast cancers across multiple lines of therapy and has also been tested extensively in HER2-positive GE-Ca [62,63].

In the pivotal international phase III Trastuzumab for Gastric Cancer (ToGA) trial, patients with unresectable or metastatic gastric or GEJ cancer were screened for HER2 overexpression using a combination of IHC and FISH [59]. Twenty-two percent (810/3665) of tumors were HER2-positive, with GEJ cancers slightly more likely to be positive than gastric cancers. In total, 584 HER2-positive patients (80% gastric and 20% GEJ) were randomized to treatment with standard chemotherapy with cisplatin and either intravenous 5-fluorouracil (5-FU) or oral capecitabine or this doublet chemotherapy combined with trastuzumab. Trastuzumab was given at 8 mg/m^2 for the first 3-week cycle and 6 mg/m^2 in subsequent cycles. After six cycles of treatment, responding patients received trastuzumab alone until disease progression. The complete RR (5%) and partial RR (42%) were modestly improved with the addition of trastuzumab, as were both PFS (6.7 months trastuzumab *vs* 5.5 months chemotherapy alone) and OS (13.8 months trastuzumab *vs* 11.1 months chemotherapy alone). Importantly, trastuzumab was generally well tolerated, and there were no differences in the toxicity profiles of the regimens including the frequency of cardiac events. A preplanned exploratory analysis showed that patients with high HER2 (defined as IHC 2+/FISH+ or IHC 3+) were the most likely subset to benefit from trastuzumab treatment. In fact, there appeared to be no benefit if IHC was 0 or 1+ regardless of FISH results. This landmark study led to the approval of trastuzumab as first-line therapy in combination with chemotherapy for patients with advanced or metastatic HER2-positive GE-Ca and has quickly become incorporated into standard practice.

The results of the ToGA trial have led to a wide range of follow-up studies. In recent years, concerns over cisplatin toxicity have led to an increased use of oxaliplatin-based regimens for patients with metastatic gastroesophageal tumors; this substitution does not appear to compromise efficacy [64]. One important question is whether alternative chemotherapy regimens can be substituted for the cisplatin/5-FU combination used in the ToGA trial. Ryu *et al.* performed a phase II study in Korean patients with metastatic or unresectable HER2-positive adenocarcinoma of the stomach or GEJ [65]. HER2-positivity was defined as IHC 2+/FISH+ or IHC 3+. Capecitabine/oxaliplatin was given on a standard 21-day cycle, and trastuzumab was given as in ToGA. Fifty-five patients were eligible for analysis. The objective RR was 68%, with the majority of these being partial responses (64%). Median PFS was 9.8 months, and OS was 21 months with 63% of patients living at least one year after starting treatment. A recently published retrospective analysis from France yielded similar results with a PFS of 9.0 months and an OS of 17.3 months [66]. These outcomes compare favorably to data from ToGA. Cross-trial comparison shows that peripheral neuropathy was the only side effect which was increased by the substitution of oxalipatin for cisplatin. These results support the common practice of substituting oxalipatin-based regimens for the cisplatin-based regimens studied in ToGA. The oral drug S-1 is an alternative fluoropyrimidine formulation that is rarely used in the West but is commonly used in Asia. It has proven efficacy in the treatment of GE-Ca, where meta-analyses suggest it is equivalent or superior to 5-FU [67–69]. Preliminary results from two single-arm phase II studies of S-1-based chemotherapy in combination with trastuzumab were recently reported in abstract form. JACCRO GC-06 tested S-1/trastuzumab in elderly patients with GE-Ca, and the WJOG7212G (T-SPACE) study tested S-1/cisplatin/trastuzumab [70,71]. In both studies toxicity was acceptable, and preliminary efficacy was similar to that seen in the studies outlined above. Therefore, it appears reasonable to replace 5-FU with S-1 in select populations. Additional studies of different chemotherapy regimens in combination with trastuzumab are ongoing including those with docetaxel and other chemotherapy combinations (NCT01295086, NCT01364493, NCT02004769 and NCT01928290). These studies should provide further data on whether the safety and efficacy of trastuzumab combinations can be expanded beyond cisplatin/5-FU.

While trastuzumab improves survival in HER2-positive GE-Ca, the results of ToGA were modest. There is theoretical concern that differences in trastuzumab metabolism between breast cancer and GE-Ca patients may mean that currently used regimens are underdosing GE-Ca patients, especially those with increased tumor burden. A published case report of a patient who responded only after an increase in the trastuzumab dose supports this concept [72], as do pharmacokinetic data collected in the ToGA trial [59]. The question of optimal trastuzumab dose is currently being addressed in the HELOISE trial (NCT01450696), a phase III study testing cisplatin/capecitabine in combination with standard-dose *vs* high-dose trastuzumab in the first-line setting

Given the success of trastuzumab in the metastatic setting, ongoing trials are determining whether it can improve cure rates for those patients with earlier stage disease. In 2007, Safran *et al.* published a phase I/II study evaluating trastuzumab in combination with chemoradiation for patients with resectable esophageal cancer [73]. The regimen was tolerable, and it appeared at least comparable to standard chemoradiation approaches. This feasibility study led to a similarly designed phase III study, RTOG 1010 (NCT01196390), which is currently evaluating the role of neoadjuvant carboplatin/paclitaxel and radiation with or without trastuzumab for patients with locally advanced, resectable HER2-positive esophageal or GEJ adenocarcinoma. Trastuzumab is given on a weekly schedule during chemoradiation and then again every three weeks, up to 13 cycles, postoperatively. A similar trial, TOXAG (NCT01748773), is adding trastuzumab to capecitabine/oxaliplatin chemoradiation.

Building on the MAGIC trial, which established perioperative chemotherapy with epirubicin/cisplatin/capecitabine (ECX) as another standard of care for resectable GE-Ca, current trials

incorporating trastuzumab into perioperative regimens are also underway [74]. The NeoHX (NCT01130337) trial testing perioperative capecitabine/oxaliplatin in combination with trastuzumab is ongoing, with the latest results after a median of 24.1 months of follow-up showing an 18-month disease free survival of 71%, a 39% clinical RR, and an 8% pathological complete response. Median PFS and OS have not yet been reached [75]. A similar study, the HER-FLOT (NCT01472029) trial, is testing perioperative 5-FU/leucovorin/docetaxel/oxaliplatin in combination with trastuzumab. While initial results are encouraging, final results are not yet available [76].

T-DM1 is an antibody-drug conjugate that combines trastuzumab with the microtubule polymerization inhibitor emtansine, which has been approved for HER2-positive breast cancer patients [63]. It is currently being tested in GE-Ca patients who have progressed on a trastuzumab-based regimen. The phase II/III GATSBY trial (NCT01641939) will compare single-agent T-DM1 to docetaxel, while the phase I/II TRAX-HER2 (NCT01702558) trial will compare T-DM1 with capecitabine *vs* T-DM1 alone. Mature data from these trials are not yet available, but they should help define the role of T-DM1 in GE-Ca.

Pertuzumab is a second mAb that targets the HER2 extracellular domain. This antibody recognizes a different epitope than trastuzumab, and studies both in preclinical models of gastric cancer and in patients with breast cancer show that combining the two HER2-targeted antibodies increases activity [77,78]. Mature results with pertuzumab/trastuzumab combinations in GE-Ca are limited. However, Bang *et al.* reported the JOSHUA (NCT01461057) study, which tested the chemotherapy/trastuzumab regimen from ToGA in combination with two different dosing schedules of pertuzumab [79]. This study had primary endpoints of safety and drug metabolism rather than clinical efficacy. However, a RR of more than 50% was seen in this small trial. The phase III JACOB (NCT01774786) trial is now ongoing and is randomizing patients to cisplatin/fluoropyrimidine with or without pertuzumab. Another related trial, INNOVATION (NCT02205047), will study trastuzumab/pertuzumab plus chemotherapy in the neoadjuvant setting.

Anti-HER2 TKIs: lapatinib and afatinib

Anti-HER2 TKIs have been tested in HER2-positive GE-Ca with mixed results. Lapatinib is a HER2-specific TKI that has been approved for HER2-positive breast cancer but has been less successful in GE-Ca [63]. The LOGiC trial was a randomized phase III trial of chemotherapy (capecitabine and oxaliplatin) combined with placebo or lapatinib in the first-line treatment of advanced GE-Ca. This trial randomized 487 HER2 FISH+ patients. IHC was also performed, but was not used to determine eligibility for the trial. The results showed a non-significant trend towards benefit in the lapatinib arm compared with the placebo (OS 12.2 *vs* 10.5 months; PFS 6 *vs* 5.4 months; RR 53% *vs* 40%, respectively). A preplanned analysis showed a benefit for Asian patients and for those < 60 years old [3,80]. This study did not improve on the ToGA study, and thus trastuzumab remains the standard of care in the first-line setting.

The role of lapatinib in the second-line setting has also been addressed. The phase III TyTAN trial randomized 261 Asian HER2 FISH+ patients, who had clinical progression on first-line chemotherapy, to receive placebo or lapatinib in combination with paclitaxel [81]. The majority (95%) of patients had not received previous HER2-targeted therapy. While those receiving lapatinib had a statistically significant increase in RR (27% *vs* 8%), there was only a non-significant trend towards benefit in survival measures (OS 11.0 *vs* 8.9 months; PFS 5.4 *vs* 4.4 months). Subset analysis suggested a benefit for IHC3+ tumors and Chinese (*vs* Japanese) patients. A similar but smaller German phase II trial (NCT01145404) randomized HER2 FISH+ patients to second-line capecitabine with placebo or lapatinib. The primary endpoint of improved RR was not achieved, and survival for both cohorts was very short [82]. Taken together, these trials show no benefit with the addition of lapatinib to chemotherapy for patients with metastatic GE-Ca in either the first- or second-line setting. There is currently no role for lapatinib outside of a clinical trial.

Afatinib is a small-molecule TKI that targets multiple members of the ErbB family including EGFR, HER2, HER3 and HER4 [83]. It is currently approved for use in lung cancer patients but is being actively studied in other cancers including HER2-positive breast and GE-Ca. Preclinical models using patient-derived HER2-positive tumor xenografts showed that afatinib treatment led to tumor regression [84]. A phase II study of afatinib for GE-Ca that had progressed on trastuzumab showed that daily afatinib led to a disease stabilization rate of 40% in this heavily treated population [84,85]. Other trials are studying afatinib in combination with trastuzumab or chemotherapy, including NCT01649271 (phase I, first-line afatinib/trastuzumab), NCT01743365 (phase II, first-line afatinib/cisplatin/5-FU), NCT01522768 (phase II, second-line afatinib/trastuzumab after progression on trastuzumab alone) and NCT02274012 and NCT02501603 (both phase II, second-line afatinib/paclitaxel after progression on trastuzumab alone).

HER2 targeted therapies: conclusions

In summary, targeted therapy for HER2-positive GE-Ca can be considered as one of the clear successes in this disease. Trastuzumab in combination with chemotherapy is currently the standard of care for HER2-positive metastatic GE-Ca, and ongoing studies will further clarify the role of trastuzumab and other mAb-based therapies in both locally advanced and metastatic disease. Additional novel approaches targeting HER2-positive disease, including the bispecific antibody MM-111, vaccines and HER2-specific chimeric antigen receptor modified T-cells, are being explored. Many trials are incorporating in-depth characterization of tumor samples (sometimes at multiple time points) to determine biomarkers that may predict response and to evaluate mechanisms of resistance. In the coming years, we will learn how HER2-targeted therapies impact earlier-stage disease, how combination approaches compare to single agents and how best to sequence available agents including how to treat HER2-positive patients who have progressed on trastuzumab. Of particular interest is whether these studies will show improvements in survival and cure that parallel those seen in breast cancer patients.

The VEGF pathway

Vascular endothelial growth factors (VEGFs) are important signaling proteins involved in both the *de novo* formation of the embryonic circulatory system and the growth of blood vessels from pre-existing vasculature. These normal functions are disrupted during cellular transformation in which aberrant VEGF pathway activity drives carcinogenesis, invasion and metastasis. While multiple VEGF ligands and receptors exist, the most important interaction in the process of angiogenesis and cancer is the binding of the ligand VEGF-A to the receptor VEGFR2 [86,87]. Ligand binding leads to receptor dimerization, which activates downstream signaling cascades that promote

angiogenesis. Multiple preclinical studies have shown that VEGF enhances tumor growth and metastasis and that inhibition of the VEGF signaling cascade results in remarkable antitumor responses [88]. In terms of prognosis, the overexpression of VEGF in GE-Ca was associated with tumor aggressiveness and with worse clinical outcomes, as have been found in colorectal cancer [89–91].

Clinical results of agents targeting VEGF pathways

Adding anti-VEGF agents to chemotherapy improves outcomes in colorectal and other cancers, thus providing a clear rationale for testing these agents in GE-Ca. A variety of agents targeting the VEGF/VEGFR pathway are available. mAb and related therapies include bevacizumab [92], ramucirumab [93] and ziv-aflibercept [94]. VEGFR TKIs studied in GE-Ca include regorafenib, sunitinib, sorafenib and apatinib.

Anti-VEGF/VEGFR mAbs: bevacizumab, ramucirumab and ziv-aflibercept

Bevacizumab is a mAb targeting VEGF-A. The preclinical and clinical efficacy of bevacizumab in combination with chemotherapy in multiple tumor types led Shah *et al.* to perform a phase II trial of irinotecan/cisplatin/bevacizumab in patients with metastatic GE-Ca [95]. The results of this study were encouraging, with approximately two-thirds of patients responding to treatment. The PFS was 8.2 months, and OS was 12.3 months, which compared favorably with historical controls. A second phase II study used a modified docetaxel/cisplatin/5-FU regimen in combination with bevacizumab and showed even better results (albeit with significant toxicities): 66% overall RR, PFS of 12 months, and OS of 16.8 months [95].

These promising phase II results prompted further evaluation of bevacizumab and chemotherapy in the phase III setting. The AVAGAST trial evaluated bevacizumab versus placebo in combination with cisplatin/capecitabine chemotherapy for first-line treatment of advanced or metastatic GE-Ca [96]. Although there was a trend toward benefit, the primary endpoint of OS was not improved (OS 12.1 months with bevacizumab *vs* 10.1 months with placebo). Interestingly, both PFS and RR were improved with the addition of bevacizumab (PFS 6.7 *vs* 5.3 months and RR 46% *vs* 37.4%, respectively). Preplanned subgroup analysis showed varying efficacy according to geographic region. While Asian patients showed no benefit, those from the Americas did, with OS increased from 6.8 months to 11.5 months with the addition of bevacizumab. Notably, OS was much better in Asian patients regardless of study arm (~12 months), possibly indicating a difference in the underlying tumor biology. Patterns of second-line therapy may also help to explain these discordant outcomes. A very similar randomized phase III trial (AVATAR) was performed exclusively in treatment-naïve Chinese patients [97]. This study confirmed a lack of benefit with the addition of bevacizumab. The OS (~11 months), PFS (~6 months) and RR (~30%) were essentially identical regardless of treatment arm.

One ongoing study of interest is the MAGIC-B study, in which bevacizumab is added to each cycle of perioperative ECX chemotherapy and then continued for an additional six courses as single-agent maintenance. An improved RR may improve R0 resection rates and ultimately impact OS and cure rates. Preliminary safety data from MAGIC-B have recently been published and show that the addition of bevacizumab does not result in unacceptable toxicity; efficacy results are not yet available [98]. While additional studies of bevacizumab and chemotherapy for advanced or metastatic GE-Ca are ongoing, the definitive negative results of AVAGAST and AVATAR do not support the use of bevacizumab outside of a clinical trial.

Ramucirumab is a mAb targeting the VEGFR2 growth factor receptor that showed activity in preclinical models of GE-Ca [93]. The REGARD trial randomized 355 advanced or metastatic gastric/GEJ patients to ramucirumab alone (8 mg/kg IV every two weeks) or placebo as second-line therapy [99]. All patients had an ECOG performance status (PS) of 0 or 1. Ramucirumab treatment significantly improved OS (5.2 *vs* 3.8 months) and PFS (4.2 *vs* 2.9 months). Although the objective RR was very low, the overall DCR was also improved at 49% for patients receiving ramucirumab *vs* 23% for placebo. The drug was tolerable with hypertension being the only significantly increased side effect. The modest improvement in OS with ramucirumab was comparable to that seen in previous studies of second-line cytotoxic chemotherapy for similar patient groups [100,101].

The hypothesis that ramucirumab combined with chemotherapy could improve on the results of REGARD was tested in the RAINBOW trial, a phase III, placebo-controlled trial that randomized 665 patients to paclitaxel combined with either ramucirumab or placebo [102]. Just as in REGARD, patients in RAINBOW benefited from the addition of angiogenesis inhibition. The OS favored the ramucirumab group (9.6 *vs* 7.4 months with placebo) as did the PFS (4.4 *vs* 2.9 months). The DCR was higher in those receiving ramucirumab as well (80% *vs* 64%). Subgroup analysis showed that patients with GE-Ca appeared to have more benefit than those with stomach cancer. Furthermore, Asian patients did not appear to benefit from the addition of ramucirumab compared with patients from other parts of the world, although there was again an excellent survival of 10–12 months, and results may have been positively influenced by the increased use of additional lines of therapy in this population. Together, REGARD and RAINBOW support the use of ramucirumab-based second-line therapy as a new standard of care. Other ongoing second-line trials will test different dose schedules of ramucirumab with paclitaxel (NCT02514551) or will combine ramucirumab with albumin-bound nab-paclitaxel (NCT02317991) to see if these modifications can improve on the results seen in RAINBOW.

Given these positive results, there is great interest in incorporating ramucirumab into earlier lines of therapy. One study performed in the United States, and published only in abstract form, evaluated the combination of 5-FU/leucovorin/oxaliplatin (FOLFOX) plus ramucirumab in untreated patients with unresectable or metastatic GE-Ca (NCT01246960) [103]. Neither PFS nor OS were significantly improved with the addition of ramucirumab. More than half of the 168 patients treated on this trial had esophageal cancer, but analyzing the results by tumor site did not markedly change the conclusion that adding ramucirumab is not indicated in the first-line setting, at least in combination with FOLFOX. The ongoing RAINFALL study (NCT02314117) is combining ramucirumab or placebo with cisplatin/capecitabine as first-line therapy for patients with GE-Ca to determine if there is synergy with this alternative chemotherapy regimen [104].

A final antibody-based strategy is the VEGF trap ziv-aflibercept [94]. This drug is an engineered molecule that combines VEGFR1- and VEGFR2-binding domains with an IgG Fc. Because it binds both VEGF receptors, there is a theoretical advantage over the agents already discussed. Positive results from the VELOUR study led to ziv-aflibercept being approved for the treatment of colorectal cancer [105]. There is an ongoing phase II randomized trial of ziv-aflibercept or placebo in combination

with FOLFOX chemotherapy for advanced and metastatic GE-Ca (NCT01747551). Given the discordant results seen with bevacizumab and ramucirumab, it will be interesting to see if ziv-aflibercept adds benefit to FOLFOX in this setting. One safety concern with ziv-aflibercept is gastrointestinal perforation, which may be more common than that seen with bevacizumab [106].

Anti-VEGFR TKIs: sunitinib, sorafenib, regorafenib, and apatinib

Small molecule TKIs represent a second group of drugs that target angiogenesis pathways. The majority of these are multitargeted and affect multiple kinases simultaneously. They have been developed as angiogenesis inhibitors due to their activity against VEGF receptors, but their promiscuous nature means the exact mechanism of action can be difficult to discern. For brevity, this review will focus only on those TKIs that have been extensively tested in GE-Ca.

Two angiogenesis inhibitors, sunitinib and sorafenib, have been found to have clinical activity in multiple cancer subtypes. Sunitinib is currently FDA approved for treatment of gastrointestinal stromal tumor (GIST), pancreatic neuroendocrine tumor (NET) and kidney cancer, while sorafenib is approved for thyroid, liver and kidney cancers. Both of these agents have been tested in GE-Ca as well but unfortunately have shown only modest activity with substantial toxicity. Sunitinib and sorafenib have failed to show significant benefit in first- or second-line treatment either as single agents or in combination with chemotherapy, although the docetaxel/sunitinib combination showed improved RR compared with chemotherapy alone (41% *vs* 14.3%) [107–115]. No phase III trials of either agent in GE-Ca are currently ongoing.

Regorafenib is an oral multi-kinase inhibitor with activity against VEGFR1-3 and TIE2 as well as other growth factor receptors. It is very similar to sorafenib and is thought to function as an angiogenesis inhibitor. It is currently approved as a single agent in end-line therapy for patients with metastatic colorectal cancer and GIST, where it provides modest benefit but significant toxicity [116–118]. The international placebo-controlled phase II INTEGRATE study (ACTRN12612000239864) randomized metastatic GE-Ca patients who had been previously treated with one to two lines of chemotherapy to regorafenib at 160 mg daily for 21 out of 28 days *vs* placebo. The results have been reported in abstract form [119]. PFS for patients on this trial was short (11.1 weeks for regorafenib *vs* 3.9 weeks for placebo), but the difference was statistically significant. The OS showed a trend towards improvement with regorafenib but did not reach significance (25 weeks for the regorafenib *vs* 19.4 weeks for placebo). Regorafenib appeared to be of more benefit to Korean patients compared with others on the trial. Other planned and ongoing trials are studying regorafenib in metastatic GE-Ca including NCT02241720 (single-agent regorafenib in the second line), NCT01913639 (regorafenib/FOLFOX in the first line), the REPEAT (NCT02406170) trial (regorafenib/paclitaxel in the second line) and NCT02234180 (adjuvant single-agent regorafenib after neoadjuvant chemotherapy and surgery for node-positive disease). The results of these studies will further define the role of regorafenib in GE-Ca but, as in colorectal cancer, current studies have yet to show a clinically meaningful improvement in survival with the use of this agent.

Apatinib is an orally available selective inhibitor of VEGFR2 with potent activity in preclinical models. A phase I trial in China suggested that it was well tolerated and of benefit for patients with pretreated metastatic GE-Ca. A follow-up phase II trial tested apatinib as a single agent in the third-line setting in patients with ECOG PS 0–1 [120]. The trial was placebo controlled, and two separate dosing schemas were used (850 mg daily or 425 mg twice daily). A total of 144 patients were enrolled on the trial. Apatinib resulted in a statistically significant increase in both PFS (1.4 months placebo *vs* 3.7 months for once-daily apatinib) and OS (3.2 *vs* 4.83 months). Most recently, a phase III study (NCT01512745) reported in 2014 confirmed these findings: apatinib at 850 mg daily as third-line therapy improved OS compared with placebo (OS of 195 days apatinib *vs* 140 days placebo) [121]. These results have led to a number of additional trials of this agent including a randomized comparison of apatinib to docetaxel in the third-line setting and two studies of apatinib maintenance (either after adjuvant chemotherapy for localized disease or after first-line therapy for metastatic disease) (NCT02510469, NCT02509806, and NCT02409199). Of note, there is little reported experience with this agent outside of China, and it is not yet clear if these results are applicable to a global population.

VEGF-targeted therapies: conclusions

Although the clinical benefit is small, the success of ramucirumab, alone or in combination with chemotherapy, in second-line therapy for GE-Ca patients represents a significant advance in targeted therapy and is now considered a standard of care in this disease. The available data on the TKI apatinib are encouraging. If they can be confirmed in additional global studies, apatinib may emerge as a standard third-line treatment for GE-Ca. The negative results seen with other agents including bevacizumab are disappointing and indeed puzzling. Here, as in other cancers, predicting the clinical efficacy of angiogenesis inhibitors remains a formidable challenge. The identification of a biomarker that predicts response to these agents could significantly improve their utility.

The MET pathway

The hepatocyte growth factor (HGF) pathway is a critical receptor tyrosine kinase-signaling network involved in the pathogenesis of GE-Ca. HGF, the only known ligand for the MET receptor, is predominantly produced by mesenchymal cells where it promotes epithelial-to-mesenchymal transition (EMT) during normal development [122]. HGF binding to MET leads to receptor dimerization and activation causing downstream signaling through RAS-MAPK, PI3K-AKT, RAC1 and FAK pathways [123]. Aberrant MET can produce a variety of cellular responses associated with cellular transformation including proliferation, motility, angiogenesis and invasion [123,124].

While missense mutations in the MET tyrosine kinase domain or juxtamembrane domain occur at a low frequency, MET overexpression or amplification is common in gastric cancers [125]. More than 50% of gastric cancers have MET overexpression by IHC, and approximately 20% have MET amplification by FISH [126]. MET amplification is associated with poor prognosis as these tumors are typically high-grade adenocarcinoma that present at advanced stages [127]. Interestingly, HER2, MET and EGFR amplifications were mutually exclusive events among 489 patients with GE-Ca, and MET amplification was the strongest predictor of poor prognosis in this study [128]. In preclinical models, amplification of MET was associated with high sensitivity to MET inhibitors [129]. Thus, there is extensive preclinical rationale for therapies targeting this pathway [124,130].

Clinical results of agents targeting the HGF/MET pathway

MET inhibition has now been extensively tested in patients with GE-Ca. Available MET inhibitors include mAbs recognizing either the ligand HGF (rilotumumab) or the MET receptor (onartuzumab) and small molecule inhibitors of the MET tyrosine kinase (AMG-337, tivantinib, crizotinib, and cabozantinib).

Anti-MET mAbs: onartuzumab and rilotumumab

Onartuzumab is a mAb that binds to the extracellular domain of the MET receptor. MetGastric (NCT01662869) was a randomized trial of FOLFOX chemotherapy in combination with onartuzumab (10 mg/kg every two weeks) or placebo as first-line therapy for patients with HER2-negative, MET-positive metastatic gastroesophageal tumors. MET expression was measured using IHC and graded as MET 1+, 2+ or 3+. Primary endpoints were OS in the total treatment group and in the subgroup of MET 2+/3+ patients. Planned enrollment was 800 patients, but the trial was closed after negative results of a phase II study in unselected HER2-negative GE-Ca (NCT01590719) [131]. The final analysis included 562 patients. The addition of onartuzumab did not improve OS (~11 months), PFS (~6.8 months) or RR (~40%) in the intent-to-treat population. In those patients with MET 2+ or 3+, there was a non-significant trend toward benefit in all three measures. However, this study is considered negative, and no additional studies of onartuzumab in GE-Ca are currently underway

Rilotumumab is a mAb recognizing the growth factor HGF. It has been evaluated in both phase II and phase III trials. NCT00719550 was a phase I/II study for previously untreated metastatic GE-Ca patients regardless of MET status [132,133]. Patients were randomized to receive standard ECX chemotherapy alone or in combination with two different doses of rilotumumab (7.5 or 15 mg/kg, given every three weeks). The addition of targeted therapy improved PFS and OS, and MET status appeared to predict response. These encouraging results led to the randomized phase III Rilomet-1 (NCT01697072) trial for patients who were treatment naïve, HER2-negative and MET-positive by IHC [134]. Rilotumumab was given at the 15 mg/kg dose. Six hundred and nine patients were randomized, but the trial was stopped early due to inferior outcomes in the rilotumumab arm. Final analysis showed that OS, PFS and ORR were worse in patients receiving rilotumumab. A similar study, Rilomet-2, is still ongoing in Asian patients [135]. Eligibility and rilotumumab dosing are as per Rilomet-1, but the chemotherapy is cisplatin/capecitabine in this trial. The results of this trial have not yet been reported. However, the manufacturer of rilotumumab has halted further development of this agent based on available negative results seen in both GE-Ca and lung cancer. Some have raised concerns that IHC is not optimal for identifying patients who may respond to MET-targeted therapies and that the reliance on IHC in these studies led to their failure. With this in mind, other planned studies of MET-targeted therapies will use IHC to look at MET protein expression coupled with FISH to measure MET gene amplification in an attempt to identify patients most likely to benefit [136].

Anti-MET TKIs; AMG 337, tivantinib, and crizotinib

While the data on onartuzumab and rilotumumab have been disappointing, other MET-targeted agents are still under evaluation and have shown promise in early studies. AMG 337 is an oral TKI, which is a potent and selective inhibitor of MET, that is being tested in many MET over-expressing tumor types. Kwak *et al.* presented early toxicity and efficacy results of AMG 337 in a subset of patients with GE-Ca [137]. Thirteen patients whose tumors showed MET amplification were treated, and eight had at least a partial response. Most responses were rapid, and occurred within four weeks of starting the drug, and a minority of responses were durable, lasting 100 weeks or longer. The drug was tolerable with headache, nausea, vomiting and fatigue as the main side effects. Additional clinical trials including NCT02016534 and NCT02096666 are testing AMG 337 in MET amplified GE-Ca patients in a non-randomized fashion. Both trials are no longer recruiting patients, but results are not yet available. There is also a planned randomized placebo-controlled phase I/II trial (NCT02344810) studying the efficacy of FOLFOX alone or in combination with AMG 337 as first-line therapy of HER2-negative, high MET-expressing gastric and esophageal adenocarcinoma. In this trial, MET will be evaluated by FISH and IHC to determine the optimal method for identifying those patients most likely to benefit from therapy.

Tivantinib is an oral MET TKI that was tested in a single-arm phase II trial (NCT01611857) in unselected metastatic GE-Ca patients in combination with FOLFOX. Preliminary results observed similar activity to historical data with chemotherapy alone. It is not yet known if measures of MET expression will identify patients with extended responses or if additional trials of this agent are planned [138].

Crizotinib is a multitargeted TKI that is approved for the treatment of ALK-positive lung cancer; this agent also inhibits the MET tyrosine kinase. Preclinical experiments have shown that crizotinib can inhibit the growth of MET-overexpressing gastric cancer cell lines and *in vivo* tumor models [139,140]. An exploratory analysis of a phase I trial of crizotinib in solid tumor patients found that two of four patients with MET-amplified GE-Ca had transient tumor shrinkage [128]. Other studies of crizotinib in GE-Ca are ongoing including NCT02435108, a pilot study testing crizotinib as third-line therapy for MET-positive gastric cancer in Korea, and NCT02034981, which will test crizotinib across multiple genetically defined tumor types including MET-amplified gastric cancer.

MET targeted therapies: conclusions

In summary, despite great promise in preclinical studies, MET-targeted approaches so far have failed to benefit patients. It will be interesting to see if TKIs will prove more successful than antibody-based therapies. The lack of a reliable biomarker that predicts response is a major hurdle that may be overcome by ongoing studies. Furthermore, translational studies have shown extensive crosstalk between MET and other RTKs that may cause inherent or acquired resistance to MET inhibitors [141–143]. Perhaps rationally designed combination therapies will improve the efficacy of MET inhibition [144]. For example, cabozantinib, which has dual activity against both MET and VEGFR2, has appeared effective in preclinical models [136,145].

Overall conclusions and future perspectives

GE-Ca remains among the most prevalent and fatal malignancies worldwide. Over the last 20 years, there has been slow but steady progress in our molecular understanding of this group of diseases. The hope was that this would translate to increased and effective use of targeted therapies in the clinic with improved patient outcomes and minimal toxicity. Unfortunately, this promise is not yet fulfilled, and we remain far from having a 'magic bullet'. Two targeted agents, trastuzumab and

ramucirumab, are currently approved for treating advanced or metastatic gastroesophageal cancers but have admittedly had modest survival benefits. These agents also augment, rather than replace, traditional cytotoxic chemotherapy, and thus they have not achieved the goal of minimizing toxicity. Finally, despite preclinical promise, the majority of targeted agents have failed in clinical trials.

To improve on these results as we move forward, we must first refine patient selection for any given therapy. Few studies prospectively incorporate biomarkers into study design. Further, our current reliance on overexpression/amplification of single receptors in isolation has failed in the majority of cases. A shift towards comprehensive molecular phenotyping of tumors should yield multidimensional information (e.g. recurrent mutations combined with gene/protein expression changes) that may better predict response to targeted therapies. It is critical that such studies include multiple biopsies in space and time, given the known tumor heterogeneity and tumor evolution. Combinations of targeted agents may simultaneously block redundant pathways and counteract resistance mechanisms. This level of phenotyping remains expensive and is not feasible for the vast majority of patients but can be expanded in the context of clinical trials.

Even with a better understanding of predictive markers and resistance mechanisms, it seems likely that targeting receptor tyrosine kinases will provide only incremental benefits over chemotherapy alone. It is hoped that these results can be augmented by ongoing and significant advances in cancer immunotherapy. Most cancers including GE-Ca exploit multiple mechanisms in order to escape immune-cell recognition and antitumor effector functions [146,147], and drugs that counter immune checkpoints have led to durable responses in patients with advanced cancers [148]. One class of agents, the PD-1 inhibitors, have been tested in advanced GE-Ca [149,150]. The preliminary data demonstrate manageable toxicity and promising antitumor activity in heavily treated patients. More rigorous phase III trials such as KEYNOTE-061 (NCT02370498), which will study PD-1 blockade with pembrolizumab *vs* paclitaxel in advanced GE-Ca, are underway. Also, early phase trials are beginning to test traditional targeted therapies in combination with immunotherapeutics (e.g. NCT02318901 (pembrolizumab plus trastuzumab) and NCT02443324 (pembrolizumab plus ramucirumab)). Perhaps these promising approaches will provide longer lasting benefits than are currently seen with targeted therapies alone.

Looking to the future, we anticipate a greater inclusion of key biomarkers into GE-Ca study design, subdividing patients not by tumor site of origin but by histologic and molecular signature. With this added information, the use of targeted therapy can be refined and better applied to precision oncology, hopefully translating into improved clinical outcome for this complex and heterogenous group of patients.

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