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Clinical impact of tumour biology in the management of gastroesophageal cancer

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Abstract

The characterization of oesophageal and gastric cancer into subtypes based on genotype has evolved in the past decade. Insights into the molecular landscapes of gastroesophageal cancer provide a roadmap to assist the development of new drugs and their use in combinations, for patient stratification, and for trials of targeted therapies. Trastuzumab is the only approved treatment for gastroesophageal cancers that overexpress HER2. Acquired resistance usually limits the duration of response to this treatment, although a number of new agents directed against HER2 have the potential to overcome or prolong the time until resistance occurs. Beyond that, anti-VEGFR2 therapy with ramucirumab was the first biological treatment strategy to produce a survival benefit in an unselected population of patients with chemotherapy-refractory gastroesophageal cancer. Large initiatives are starting to address the role of biomarker-driven targeted therapy in the metastatic and in the perioperative setting for patients with this disease. Immunotherapy also holds promise, and our understanding of subsets of gastroesophageal cancer based on patterns of immune response continues to evolve. Efforts are underway to identify more relevant genomic subsets through genomic screening, functional studies, and molecular characterization. Herein, we provide an overview of the key developments in the treatment of

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gastroesophageal cancer, and discuss potential strategies to further optimize therapy by targeting disease subtypes.

Oesophageal and gastric cancers are a global health problem, with 1,417,000 newly diagnosed patients annually, and 1,123,000 annual deaths from these diseases[1]. The incidence and geographical distribution of gastroesophageal cancer varies: noncardia gastric cancer is more prevalent in East Asia, Central-East Europe, Latin America, and Africa, whereas adenocarcinomas of the distal oesophagus, the gastroesophageal junction (GEJ) and the proximal stomach are more prevalent in Western Europe, North America, and Australia[2,3]. Gastroesophageal cancers are aggressive and often spread to distant organs early in the disease trajectory.[4] In general, around 16–37% of cancers that reach the submucosal layer (T1b category) have already spread to locoregional lymph nodes[5,6]. In the Western hemisphere, most patients present with locally advanced or metastatic disease, which mandates the use of systemic chemotherapy, either perioperatively or in the palliative setting.

For patients with gastroesophageal cancer that is not amenable to complete resection owing to metastatic disease, palliative chemotherapy can prolong survival, and improve symptoms and quality of life compared with best-supportive care (BSC) alone[6]. Chemotherapy combinations comprising platinum compounds (for example, oxaliplatin, or cisplatin) and fluoropyrimid ines (5-fluorouracil (5-FU), capecitabine, or S-1) are more effective than fluoropyrimidine monotherapy in the first-line setting[7]. The addition of a third chemotherapy agent — docetaxel or epirubicin — in patients with an excellent functional and nutritional status with uncompromised organ functions can improve disease control and tumour response rate, which translates to a very modest overall survival benefit when compared with doublet therapy[8,9]. Thus, for most patients, the use of chemotherapy doublets is preferred, owing to a more-favourable toxicity profile and risk–benefit ratio.

Indeed, treatment consisting of a two-drug chemotherapy backbone is often used in practice because targeted therapies are increasingly implemented in the therapeutic algorithm of gastroesophageal cancer. HER2 and VEGFR2 are clinically validated molecular targets in the treatment of advanced-stage gastroesophageal cancer. Trastuzumab (a HER2-targeting monoclonal antibody) and ramucirumab (an anti-VEGFR2 antibody) are considered the standard-of-care treatments for metastatic gastroesophageal cancer[10–12], but the availability of these drugs differs among countries. A recently proposed treatment algorithm, based on national and international guidelines and on our interpretation of the latest published data, is shown in FIG. 1 (REFS 7,13–15).

Patients require new treatment options, particularly when standard therapies are exhausted. Despite advances in the treatment of gastroesophageal cancer, predicting which tumours will become resistant to therapy remains challenging; however, with a greater understanding of molecular classifications of gastroesophageal cancer subtypes, we hope to improve patient selection for biological therapy, which should enhance therapeutic benefit and outcomes. Herein, we provide a synopsis of the current awareness of the unique biology of gastroesophageal cancer and discuss the clinically applicability of these findings.

Biology of gastroesophageal cancer

Historically, classification of gastric adenocarcinoma was performed on the basis of histological and clinical characteristics. The key subtypes included: Lauren's diffuse type that encompassed the signet ring type gastric cancer[16], with higher propensity for a intraperitoneal metastasis pattern[17] and silencing of *CDH1* (REF. 18); distal gastric cancer-intestinal type, arising from precursor lesions in the setting of atrophic gastritis and chronic inflammation due to *Helicobacter pylori*[19–21]; and gastric cardia and GEJ-related to inflammation resulting from gastric acid reflux and lifestyle factors, such as obesity and smoking[22,23]. Stage for stage, gastric cardia and GEJ tumours have a worse prognosis compared with nondiffuse distal tumours[24], and have the highest incidence of *HER2* (*ERBB2*) amplification[10,25,26]. The incidence of distal gastric cancer is decreasing worldwide, but GEJ tumours are rapidly increasing in incidence in the Western hemisphere[27] – most rapidly in the USA, and particularly in young males aged 25–39 years[21].

H. pylori is a group 1 carcinogen according to the WHO because infection with this bacteria can lead to gastric cancer[28]; although billions of people worldwide are infected with *H. pylori*, fortunately <1% will develop gastric cancer[29]. This low penetrance of gastric cancer in infected individuals is probably the result of the interplay between *H. pylori* virulence factors (vacAs1, vacAm1, and cagA) and certain genetic polymorphisms (IL-1B-511*T carriers: IL-1B-511*T/*T or IL-1B-511*T/*C)[30]. Furthermore, obesity[31], smoking and alcohol consumption, a diet high in salt[32], and low cereal fibre[33] and vege table[34] consumption increases the risk of developing gastroesophageal cancers.

Epstein–Barr virus (EBV) infection should be suspected in gastric adenocarinomas that have a characteristic lymphoid infiltrate. This finding suggests that focal EBV infection can occur before neoplastic transformation[35]. These infiltrates occur in ~5% of tumours, predominantly in men, and stage for stage, are associated with a more-favourable prognosis, despite the fact that most of these tumours are proximally located, which usually portends a worse prognosis to distally located tumours[36].

Genomics of gastroesophageal cancer

Multiple groups have used genomic analysis technologies, such as large-scale genome sequencing, to accelerate our understanding of the molecular basis of gastroesophageal cancer. The Cancer Genome Atlas (TCGA) published a comprehensive molecular characterization of gastric cancer[37], in which they evaluated mutations, gene copy-number changes, gene expression, and DNA methylation across 295 patients with gastric cancer. Historically, this disease has been viewed as a single entity, but data from the TCGA unbiased informatics approach that integrated somatic genomic alterations, methylation status and gene-expression analysis has redefined the disease into four distinct subclasses. First, tumours with EBV infection showed profound hypermethylation, and 80% of these tumours harboured a *PIK3CA* mutation. Second, tumours with microsatellite instability (MSI) had DNA hypermethylation (with patterns distinct from that observed of EBV-positive tumours) and had elevated somatic mutation rates, with highly recurrent mutations

of *PIK3CA* (42%) and *ERBB3* (26%) — with 12% of tumours having alterations of both genes. Third, tumours with chromosomal instability (CIN) showed marked aneuploidy, and although they lacked common mutations in *PIK3CA* and/or *ERBB3*, recurrent amplifications of receptor tyrosine kinase (RTK) genes were observed, most notably *HER2* (24%). Finally, tumours lacking aneuploidy and elevated rates of mutation or hypermethylation were termed genomically stable (GS), and were mainly represented in the diffuse histological subtype. The TCGA identified that 30% of these GS tumours harboured novel alterations in components of the Rho signalling pathway, particularly somatic mutations of *RHOA* or fusion genes involving Rho-GTPase activating proteins (FIG. 2).

Comprehensive molecular profiling is feasible in routine clinical practice using metastatic tumour specimens. At the Memorial Sloan Kettering Cancer Center (MSKCC) next-generation sequencing and gene-copy-number analysis is routinely performed on all advancedstage gastric tumours, in line with the institutional protocol. Archival formalin-fixed, paraffin embedded (FFPE) samples are analysed using the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT™) assay, an on-site 410 cancer-associated-gene bait capture, next-generation sequencing (NGS) assay. The assay is capable of identifying point mutations, small insertion or deletion events (indels), and gene-copy-number aberrations in cancer-associated genes[38]. Data is reported in the clinical medical record and maintained in the MSKCC internal cBioPortal for Cancer Genomics[39], a web-based resource for exploring, visualizing, and analysing multidimensional cancer genomics data[40]. A comparison of the MSKCC data with the TCGA results revealed an over-representation of the CIN subtype (65% versus 50% in TCGA). We found very few EBV-associated or MSI-subtype tumours (approximately 3% each), with the remaining 29% being chromosomally stable[41].

The Asian Cancer Research Group (ACRG) analysed 300 primary gastric tumours using targeted sequencing, genome-wide copy-number data and gene-expression data, and described four molecular subtypes linked to distinct clinical outcomes and prognosis[42]. Their mesenchymal-like type tumours (microsatellite stable (MSS/epithelial-to-mesenchymal transition (EMT) pheno type) includes diffuse-subtype tumours with the worst prognosis, which have a tendency to occur at an earlier age, and have the highest frequency of recurrence (63%) of the four subtypes[42]. The ACRG MSI subtype comprises hypermutated intestinal-subtype tumours with the best overall prognosis and the lowest frequency of recurrence (22%) of the four subtypes[42]. Finally, both the p53 (*TP53*)-active (MSS/TP53+) and TP53-inactive (MSS/TP53-) subtypes include patients with intermediate prognosis and recurrence rates (with respect to the other two subtypes), with the *TP53*-active group showing a better prognosis than the *TP53*-inactive group[42]. The comparison of the ACRG subtypes with the TCGA genomic subtypes revealed similarities, such as a subset of tumours with MSI, and demonstrated that TCGA GS, EBV-positive, and CIN subtypes were enriched in ACRG MSS/EMT, MSS/TP53+ and MSS/TP53– subtypes, respectively[42]; however, the investigators observed several differences in terms patient demographics of the cohorts, molecular mechanism, driver gene, and prognosis associations[42]. An important difference was that the tumours classified as the CIN subtype by TCGA were present across all ACRG subtypes in the TCGA data set[42]. Also, tumours classified as the GS subtype in the TCGA set were present across all ACRG subtypes[42]. The ACRG describe a substantially

lower percentage of Lauren's diffuse-subtype cases in the TCGA cohort (24% in TCGA versus 45% in ACRG), with the majority (57%) of Lauren's diffuse-subtype cases classified in the TCGA GS subgroup, compared with only 27% of cases in the similar ACRG MSS/EMT subtype[42]. This finding indicates more heterogeneity in the diffuse subtype tumours included in the ACRG cohort. Other significant differences were seen with regard to the prevalence and distribution of *CDH1* and *RHOA* mutations among the different subtypes. The observed differences in prognosis of the ACRG subtypes seem to be unique to this classification system; when the investigators classified ACRG tumours using the TCGA genomic scheme they demonstrated a much weaker association with the prognosis trend[42]. In summary, the ACRG subtype classification complements the TCGA stratification approach, and supplements it by incorporating two key molecular mechanisms related to TP53 activity and EMT, in order to further stratify patients with gastric cancer.

Collectively, these efforts to further characterize gastric tumours have created a roadmap for patient stratification and genome-guided trial development. These classifications create a foundation to develop rational therapeutics for distinct groups of patients. One remaining limitation of all approaches could be the inherent heterogeneity of gastric tumours. One of the foreseeable hurdles for the use of molecular signatures in the clinic might also relate to health-care costs. Multiplexed assays could be developed and applied in gastric cancer, although immunohistochemistry and RNA *in situ* hybridization techniques could be used. Importantly, the signatures need to be validated in future prospective clinical studies and their clinical relevance needs to be confirmed in trials assessing biologically targeted drugs.

Gastroesophageal PDX programme

Most preclinical studies of gastric cancer have relied on cell lines. TCGA data for gastric cancer[37] reveal that the widely available cell lines have several intrinsic limitations, including loss of the characteristics of the parental tumour, lack of clinical annotation, and the limited representation of genotypes and subtypes. At the MSKCC and other centres, efforts are underway to establish patient-derived xenografts (PDXs) as accurate models of heterogeneous gastroesophageal tumour biology. Once PDXs are established, patient DNA samples from normal and tumour tissue, and PDX tumour DNA are analysed with a next-generation sequencing to identify mutations, small insertion and/or deletions, and structural copy-number aberrations. Primary and PDX tumours exhibit similar histology, mutational profile, and copy-number profiles, suggesting that PDXs represent the tumours from which they were derived[43]. In contrast with traditional cell lines, new PDX models that have never been in cell culture and passaged a few times, can be readily annotated for various clinical features, and seem to cluster with the TCGA cancer subtypes[43].

Hereditary predisposition

Approximately 3% of gastric cancer cases arise in the setting of hereditary diffuse gastric cancer (HDGC)[44]. A substantial portion of families with HDGC have germline inactivating mutations in *CDH1*, encoding E-cadherin, with 80% penetrance and a very high risk of developing diffuse gastric cancer[45,46]. In addition, women with a *CDH1* mutation have approximately a 40% risk of developing lobular breast carcinoma[44,47,48]. Familial gastric

cancer is now classified into '*CDH1*-positive' and '*CDH1*-negative' tumours[49]. Additional cancer syndromes associated with gastric cancer are Lynch syndrome II (hereditary nonpolyposis colon cancer type II; mostly *MLH1* and *MSH2* mutations), adenomatous polyposis coli (*APC* mutations), mutations in *BRCA2* (REF. 50), Li–Fraumeni syndrome (*TP53* mutations), and Peutz–Jeghers syndrome (*STK11* mutations)[51–54]. In 2012, gastric adenocarcinoma and proximal polyposis of the stomach (GAPPS), a new autosomal dominant syndrome, was described[55]. GAPPS is a unique gastric polyposis syndrome associated with a considerable risk of gastric adenocarcinoma. This syndrome is characterized by the autosomal dominant transmission of fundic gland polyposis, including areas of dysplasia or intestinal-type gastric adenocarcinoma, restricted to the proximal stomach, and with no evidence of colorectal or duodenal polyposis or other heritable gastrointestinal cancer syndromes[55]. Important summaries of the current knowledge about familial gastric cancer syndromes were published elsewhere in 2015 (REFS 56,57).

The vast majority of gastric cancers occur sporadically and an association exists between increased gastric cancer risk and blood group — those with blood group A have a ~20% higher risk of gastric cancer than those with groups O, B or AB[58,59], indicating increased vulnerability to environmental stressors or an associ ation with genes linked with the blood-group antigens. Furthermore, clustering of *H. pylori* infection[59] might explain the increased rate of gastric cancer in some families.

HER2-positive disease

Prevalence and prognosis

In clinical practice, the HER2 was the first, and only, membrane-bound RTK to be successfully targeted for the treatment of patients with gastric cancer[60]. In a cohort of 3,665 patient samples, the Trastuzumab for Gastric Cancer study (ToGA) revealed that 810 (22%) were HER2-positive according to predefined immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) criteria[10]. Cancers located at the GEJ have a higher rate of HER2 positivity than distal gastric cancers[10]; intestinal cancers, according to Lauren's classification, display a much higher expression level of HER2 compared with diffuse cancer subtypes[10].

In several studies from different regions of the world, the incidence of HER2 positivity in gastric cancer was reported to be lower than in ToGA, ranging from 8–18%[26,61–65]. Although *HER2* amplification seemed to be associated with a worse prognosis in one study[66], larger investigations could not confirm HER2 protein expression or gene amplification as an independent prognostic factor[67–69]. In addition, *HER2* status in pretreatment biopsies did not predict enhanced benefit from epirubicin-platinum-fluoropyrimidine chemotherapy[70].

Testing for HER2

Accurate testing for HER2-positive status is now mandatory to identify patients with gastric cancer who will respond to trastuzumab treatment. Nevertheless, the diagnostic applicability of HER2-positivity remains challen ging in gastric cancer, owing to considerable

intratumoural heterogeneity (FIG. 3). This intratumoural heterogeneity can lead to sampling errors[26,63]. Matched biopsy and resection specimens of gastric and gastroesophageal adenocarcinoma, nevertheless, show high concordance with regard to HER2 status[71]; however, a minimum of five biopsies is recommended for reliable HER2 assessment. This relatively high number of biopsies is needed to avoid the sampling errors related to the intratumoural heterogeneity of this biomarker[63]: increasing the number of biopsies results in a decreased chance of a false-negative result. Endoscopists should be aware that smaller sample sizes might decrease the accuracy in selecting patients eligible for anti-HER2 therapy[72–75]. Besides these sampling issues, educational programmes have helped to reduce inter observer differences between pathologists and provide better information regarding patient selection for treatment[76].

Trastuzumab in stage IV gastric cancer

ToGA showed a significant overall survival benefit for patients with HER2-positive advanced-stage gastric cancer who were treated with trastuzumab and cisplatin–fluoropyrimidine (5-FU) chemotherapy. Median overall survival was 13.8 months (95% confidence interval (CI) 12–16 months) in those assigned to trastuzumab plus chemotherapy compared with 11.1 months (10–13 months) in those assigned to chemotherapy alone (hazard ratio 0.74; 95% CI 0.60–0.91; $P = 0.0046$)[10]. In many countries, trastuzumab in combination with cisplatin and 5-FU or capecitabine is now a preferred treatment option for patients with HER2-positive gastric cancers on the basis of data published in the ToGA study (FIG. 1).

ToGA showed a clear association of the HER2 protein immunoreactivity score and the benefit from treatment with trastuzumab, whereas the role of FISH in detecting any form of *HER2* amplification (defined as a *HER2:CEP17* ratio ≥2) seemed less important in relation to outcome[10]. By contrast, Spanish investigators found the level of *HER2* amplification in a cohort of 90 patients with advanced-stage gastric cancer to be significantly predictive of sensitivity to trastuzumab-based chemotherapy and overall survival. A mean HER2/CEP17 ratio of 4.7 was identified as the optimal cut-off value discriminating sensitive and refractory patients ($P = 0.005$). Similarly, the optimal cut-off for predicting survival longer than 12 months was 4.45 ($P = 0.005$), and for survival longer than 16 months was 5.15 ($P = 0.004$)[77]. Of note, identification of patients eligible for trastuzumab treatment is a demanding task. HER2 testing in gastric cancer differs from testing in breast cancer because of inherent differences in tumour biology; gastric cancer more frequently shows heterogeneity and focal staining of HER2 and incomplete membrane staining (FIG. 3)[78].

HER2-directed treatments in different settings

Despite a solid preclinical rationale[79], the RTK inhibitor lapatinib did not show significant antitumoural efficacy in HER2-positive advanced-stage gastric cancers in two clinical phase III studies[80,81]. The primary end point was not met in either study; however, subgroup analysis provided proof-of-concept for some activity of lapatinib in patients with *HER2*-amplified cancers. The problem of post-progression treatment of HER2-positive advanced-stage gastric cancers is currently unresolved; a phase III study in Asian patients and a randomized phase II study in European patients did not demonstrate that lapatinib is an

effective treatment in advanced-stage gastric cancer in patients with disease progression following treatment with trastuzumab[80,82]. In view of the fact that lapatinib monotherapy was ineffective as a salvage treatment in trastuzumab pretreated patients with HER2-positive advanced-stage breast cancer[83], the European gastric cancer study tested both lapatinib monotherapy and lapatinib in combination with chemotherapy (capecita bine); nonetheless, in both arms response rates were below the predefined threshold of clinical activity[82].

Pertuzumab is an antibody that binds to HER2 and inhibits its dimerization with other HER receptors, which is hypothesized to result in slowed tumour growth. When combined with trastuzumab, pertuzumab substantially enhanced the antitumour activity of treatment in HER2-positive human gastric cancer xenograft models[84,85]. On the basis of pharmacokinetic and safety data, a 840 mg pertuzumab dose given every 3 weeks has been selected for investigation in a phase III study of pertuzumab, trastuzumab and chemotherapy in the first-line setting of patients with HER2-positive advanced-stage gastric cancer[85,86].

Trastuzumab emtansine (T-DM1; also known in the USA as ado-trastuzumab emtansine) is an antibody–drug conjugate consisting of the monoclonal antibody trastuzumab linked to the cytotoxic agent DM1; this agent is currently being investigated in the second-line treatment of advanced-stage gastric cancer[87]. However, T-DM1 failed to prolong survival compared with taxane treatment in this study, according to data presented early in 2016 (REF. 87).

Of note, the value of targeting HER2 is almost undetermined in the perioperative setting. Two phase II studies, one from Spain[88] and one from Germany[89], reported the feasibility of neoadjuvant platinum–5-FU-based chemotherapy with trastuzumab, and reported interesting response rates: 36 patients were included in the Spanish study, and showed preoperative capecitabine/oxaliplatin-trastuzumab was feasible. An R0 resection was achieved in 28 patients (78%) and a histopathological complete response was observed in three patients (8.3%).[88] The German study[89] reported histopathological complete responses in 10 out of 45 patients (22.2%) with HER2-positive locally advanced gastric cancer who received the combination of trastuzumab and standard chemotherapy. This finding raises hope that high response rates can be achieved with trastuzumab plus chemotherapy combinations in the neoadjuvant setting and might translate into improved survival rates. This hypothesis needs to be confirmed by prospective randomized studies.

Indeed, this research gap will be closed by the ongoing European Organisation for Research and Treatment of Cancer (EORTC) INNOVATION study (NCT02205047)[90], in which investigators are randomly allocating patients with stages Ib–III gastric and GEJ cancers to receive neoadjuvant chemotherapy alone, chemotherapy plus trastuzumab, or chemotherapy plus trastuzumab and pertuzumab. The Radiation Therapy Oncology Group (RTOG) 1010 study is an ongoing phase III trial (NCT01196390)[91], in which researchers are evaluating the addition of trastuzumab to trimodality treatment (radiation therapy, paclitaxel, and carboplatin) in patients with HER2-positive oesophageal adenocarcinoma, including GEJ cancer. In addition, in East Asia, the Japanese Cooperative Oncology Group (JCOG) 1301 ran domized phase II study is underway to assess systemic chemotherapy with and without trastuzumab, followed by surgery in patients with HER2-positive advanced-stage gastric or GEJ adenocarcinoma with extensive lymph-node metastasis[92].

Resistance to anti-HER2 treatment

Accumulating evidence supports the benefits of continuing trastuzumab beyond disease progression in patients with HER2-positive breast cancer[93,94]. The results of these studies suggest that continuing trastuzumab after progressive disease in gastroesophageal cancer is a viable option; however, large prospective studies in patients with gastroesophageal cancer are needed to confirm this approach. Furthermore, how resistance against HER2-directed treatment occurs and how this could be managed are important questions. Some concepts can be gleaned from research in patients with breast cancer, but in patients with gastric cancer, much less is known about potential resistance-defining alterations, such as *PIK3CA* mutations, HER3 dimerization, upregu lation of SRC activity and *PTEN* loss[95]. Loss of HER2 expression occurs in approximately one-third of patients with HER2-positive gastric cancer treated with trastuzumab, and presents a possible mechanism for trastuzumab resistance. Upon tumour progression, molecular alterations have been observed in *EGFR* (13%), *TP53* (92%), cell-cycle mediators, such as cyclin-dependent kinases (42%) and in the PI3K/AKT/mTOR axis (21%).[96] These data suggest the need for repeat biopsies to accurately determine the appropriate use of HER2-directed therapy upon tumour progression[96].

Monitoring of anti-HER2 treatment

The variation in trastuzumab biodistribution owing to tumour burden and individual patient metabolism is well recognized as a reason for incomplete responses in patients with HER2-positive breast cancer[97,98]. Zirconium-89 (^{89}Zr)-trastuzumab PET enables non-invasive simultaneous assessment of HER2 levels in both the primary tumour and all sites of metastases[99,100]. Thus, ^{89}Zr-trastuzumab PET might help to elucidate the molecular basis of resistance to trastuzumab in patients with gastroesophageal cancer and facilitate the development of an optimal dose and schedule of HER2-targeted agents tailored to the tumour burden in individual patients.

In a preclinical study in xenografted mice[101], the use of the PET imaging tracer ^{89}Zr-trastuzumab to specifically delineate HER2-positive gastric cancer and to monitor the pharmacodynamic effects of the anti-HER2 agent afatinib was assessed and proved to be feasible. These findings need to be confirmed in prospective studies in patients treated with anti-HER2 therapy for HER2-positive gastric cancer.

Targeting other RTK signalling pathways

Targeting other RTKs with monoclonal antibodies or small-molecule inhibitors has not led to compelling efficacy thus far in patients with gastric cancer. The reasons for this lack of efficacy are various, ranging from uncertainty about the relevance of the respective signalling pathways for the progression of advanced-stage gastric cancer to appropriate patient selection for specific targeted treatments. Disappointingly, anti-EGFR directed treatment with the monoclonal antibodies cetuximab and panitumumab did not provide survival benefit in two large randomized phase III studies, despite promising data from phase II studies[102–105]. Additionally, erlotinib and gefitinib were found to be ineffective in randomized trials that compared these agents to BSC in patients with oesophageal and GEJ

cancers[106,107]; however, retrospective biomarker analysis suggests that a subpopulation of patients with tumours harbouring *EGFR* amplifications or copy-number gains might bene fit from anti-EGFR therapy[102,103,108]. Notably, *EGFR* amplifications seem to be rare in gastroesophageal cancers (occurring in <10% of patients)[102,108–110], and prospective studies with EGFR inhibitors in a patient population enriched for *EGFR*-amplified tumours are currently lacking.

Negative results have also been reported regarding targeting the HGF/MET axis. Despite promising observations with the anti-HGF monoclonal antibody rilotumumab in a randomized phase II study[111,112], two phase III studies[113,114], one testing rilotumumab, and the other onartuzumab (an antibody that inhibits HGF binding to MET), failed to reach their primary end points and did not lead to an improvement in overall survival. These failures were independent of the intensity of MET staining in patient samples[113,114]. Alternative biomarkers to guide patient selection other than MET status by IHC might be required for the promise of anti-HGF/MET treatments in gastric cancer to be realized; one such option is *MET* amplification, which might be a more-appropriate biomarker to select for tumours that will respond to MET inhibitors. Preliminary data showed that some patients with *MET* amplification (which occurs in <5% of gastric cancers[115]) can achieve complete responses with MET inhibitors, such as crizotinib or AMG337 (REFS 110,116).

To date, FGFR has not been as well explored as a therapeutic target in gastric cancer. Nevertheless, a comprehensive survey of genomic alterations in gastric cancer samples revealed *FGFR* amplification in a distinct and molecularly exclusive subgroup of patients[109]. Importantly, *FGFR*-amplified gastric cancers had a worse prognosis following curative resection[109], indicating that *FGFR* amplification may be a genetic ‘driver’ alteration. The authors, therefore, postulated that FGFR inhibitors might be useful in targeting *FGFR*-amplified tumours[109]. Activation of the FGFR2 pathway was found to be required to drive growth and survival of gastric cancers carrying *FGFR2* amplifications, both *in vitro* and *in vivo*[117]; in the TCGA gastric-cancer dataset, 9% of CIN tumours and 8% of GS tumours harboured *FGFR2* amplification[37]. A randomized phase II study raised doubt about the value of targeting FGFR1-3, however, at least with the experimental drug AZD4547 (REF. 118). AZD4547 binds to and inhibits FGFR, which may result in the inhibition of FGFR-related signal transduction pathways, and was well-tolerated. In the phase II trial of this agent, however, the analysis of progression-free survival did not reveal any statistically significant difference in favour of the AZD4547 arm, compared with the paclitaxel arm, in patients with *FGFR2*-amplified or *FGFR2*-polysomy tumours selected by FISH[118]. Reasons for the lack of efficacy of AZD4547 could be the marked intratumour heterogeneity of *FGFR2* amplification, and the low concordance with elevated FGFR2 protein expression, as exploratory biomarker analysis revealed[118]. The results of ongoing studies examining other FGFR pathway inhibitors and different biomarker-based patient selection strategies, such as *FGFR2* copy number in cell free plasma DNA, should be awaited before we abandon these agents[119].

Finally, inhibition of mTOR, a downstream signalling component of these RTK pathways, also failed to provide survival benefits in a phase III study[120]. Specifically, second-line treatment with everolimus, a small-molecule inhibitor of mTOR, did not improve overall

survival of patients with advanced-stage gastric cancer, compared with placebo[120]. Of note, patients had not been preselected by any RTK expression analysis or any other biomarker. Treatment with everolimus failed irrespective of the type of first-line chemotherapy (fluoropyrimidines, platinum agents or taxanes), according to preplanned subgroup analyses[120].

Targeting angiogenesis

Altered angiogenesis is a typical feature of neoplasia and hallmark of cancer, and targeting tumour angiogenesis is a well-established therapeutic approach[121,122]. Many key elements of tumour angiogenesis are targets of available drugs and investigational compounds[123] (FIG. 4).

Anti-VEGFR2 therapy is the first biological strategy in an unselected patient population to be associated with a survival benefit in patients with chemotherapy-refractory gastroesophageal cancer[11,12]. Ramucirumab, a fully human monoclonal IgG1 antibody targeting VEGFR2, was investigated in patients with advanced-stage gastric cancer following disease progression during or after first-line chemotherapy. Ramucirumab monotherapy and BSC were more effective than placebo and BSC in this trial[11], leading to an increase in median survival from 3.8 to 5.2 months (hazard ratio (HR) 0.776, 95% CI 0.603–0.998; $P = 0.047$). Adverse events reported in the ramucirumab were in the same range in type as well as in frequency as in the BSC arm, except ramucirumab was more-frequently associated with arterial hyper tension (a class effect of this agent and one that is typical side effect of antiangiogenic drugs), but this effect did not negatively impact quality of life[11]. The efficacy of ramucirumab in treating patients with advanced-stage gastric cancer in the second-line setting was in the same range as for single-agent chemotherapy (TABLE 1); however studies that compared second-line chemotherapy with BSC or a taxane with irinotecan all showed higher clinically significant adverse-event rates in the chemotherapy treatment arms, and typical irinotecan-related or taxane-related adverse effects[124–127]. On the basis of these observations, ramucirumab could be a preferred treatment option in patients who have previously received chemotherapy, at least when chemotherapy-related adverse events, such as alopecia, nausea, diarrhoea, infection or sensory neuropathy, need to be avoided. Of note, the anti-VEGFR2 inhibitor apatinib showed similar efficacy to ramucirumab in a randomized phase II study[128]. In the RAINBOW study, ramucirumab was investigated in combination with weekly paclitaxel following a lack of response to first-line platinum and fluoropyrimidine therapy[12]. Overall survival was significantly longer in the ramucirumab plus paclitaxel group than in the placebo plus paclitaxel group (median 9.6 months versus 7.4 months; HR 0.807 (95% CI 0.678–0.962), $P = 0.017$)[12]. The combination of ramucirumab and paclitaxel seems to be the most-effective current treatment regimen for patients with advanced-stage gastric cancer in whom disease progresses following first-line therapy. On the basis of these findings, a new treatment algorithm for second-line advanced-stage gastric cancer has recently been proposed[13] (FIG. 1).

Sorafenib, a small-molecule inhibitor that targets VEGFR2, PDGFR, RET, FLT3 and RAF1[129,130], has been demonstrated to result in disease stabilization and encouraging

progression-free survival outcomes in patients with chemotherapy-refractory oesophageal and GEJ cancer[131]. Regorafenib, which targets several receptors, including VEGFR2, also showed enhanced antitumour activity compared with placebo in a randomized phase II study in patients with gastroesophageal cancer after failure of first-line or second-line chemotherapy[132].

These data suggest an important role of angiogenesis in the progression of gastroesophageal cancer, although, in the first-line setting, three trials exploring chemotherapy in combination with either bevacizumab[133,134] or ramucirumab[135] failed to meet their primary end points. These negative results indicate that, at least in a first-line setting, inhibiting VEGF alone might not be sufficient, and inhibition of multiple compensatory pathways, such as PDGF and FGFR signalling, might be important. Indeed, preclinical models suggest that upregulation of the PDGF and FGFR pathways provide alternate escape mechanisms that drive disease progression during VEGF–VEGFR blockade[136,137]. Despite these findings, a further phase III randomized trial of ramucirumab or placebo in combination with cisplatin and capecitabine in the first-line setting in HER2-negative gastric cancer has been initiated (NCT02314117)[138].

In a subgroup analysis, high plasma VEGF-A levels and low baseline tumour expression of neuropilin-1 were suggested to be predictive of sensitivity to bevacizumab in Western patients[139]. Whether this observation is attributable to other regional treatment patterns, to drug susceptibility issues in different patient populations, or to differences in tumour biology remains to be elucidated. Unfortunately, a biomarker-based selection of patients who might benefit from antiangiogenic treatment is not possible at present.

Targeting cancer stemness

Traditionally, cancer cells within a single tumour have been considered as a homogeneous cell population until relatively late in the course of tumour progression, when hyperproliferation and genetic instability lead to distinct molecular subpopulations. In recent years, however, evidence indicates the existence of intratumoural heterogeneity and a hitherto-unappreciated subclass of neoplastic cells within tumours, termed cancer stem cells (CSCs)[122]. The CSC concept emerged in the mid-1990s, when stem-cell biologists from the University of Toronto reported that they had isolated rare human leukaemic cells that could initiate leukaemia in immunodeficient mice[140,141]. Other teams subsequently reported finding CSCs in nonhaematological cancers[142–144], including gastric cancers[145]. These cancer cell subpopulations were suggested to evade chemotherapy and radiation, partly because most treatments kill rapidly dividing cells, and CSCs proliferate and divide more slowly than other malignant cells. These CSCs could later regenerate the original tumour or metastasize in other organs[146]. Thus, the concept of combining novel CSC-directed therapies with conventional cytoreduction was postulated with the goal to achieve complete tumour eradication.

The JAK/STAT3 signalling pathway is well known for its role in tumour cell proliferation, survival, invasion, and immunosuppression: activation of this pathway promotes cancer through stem cells and inflammation, among other mechanisms[147]. STAT3 regulates mitochondrial functions, as well as gene expression through epigenetic mechanisms[148]. In

gastric cancer, especially in the diffuse subtype (according to the Lauren classification), activation of STAT3 is associated with EMT and resistance to treatment[149]. BBI608, a small-molecule inhibitor of *STAT3* gene transcription, inhibited 'stemness' gene expression and killed highly tumorigenic and metastatic cancer cells isolated from a variety of cancer types[150]. Moreover, cancer relapse and metastasis were effectively blocked by BBI608 in immunosuppressed mice[150]. A phase III study[151] is now enrolling patients with advanced-stage gastroesophageal adenocarcinoma with disease progression on previous chemotherapy. In this trial, patients will be randomly assigned to receive paclitaxel plus BBI608 or placebo, and a prolongation of overall survival is the primary end point[152].

Immunotherapy

Evading immune destruction is a recognized hallmark of cancer[122]. Targeting of immune checkpoints and agonists of T-cell activation in melanoma and lung cancer have made their way into clinical practice, although data in gastroesophageal cancer remain immature and immuno therapy should only be used in the framework of a clinical trial[152]. Nevertheless, oesophageal and gastric cancers might be excellent candidate diseases for immuno therapy, in view of the abundant somatic mutations found in these tumours, which might make the cancer cells more susceptible to recognition by the immune system[152] (FIG. 5), owing to neoepitope presentation on their surfaces that enhances tumour immunogenicity[153].

Results from two phase II studies with data presented in 2015 raised hope that a subgroup of patients with advanced-stage oesophageal and gastric cancers will bene fit from therapies targeting programmed cell-death protein 1 (PD-1)[154,155]. In one of the two studies[154], 23 patients with squamous-cell carcinoma (SCC) or adenocarcinoma of the oesophagus or GEJ showed promising responses to 10 mg/kg pembrolizumab every 2 weeks. The patients had PD-1 ligand 1 (PD-L1) expression on ≥1% of cells in tumour nests or PD-L1-positive stromal bands determined centrally by IHC; they had not responded to standard therapy; had ECOG performance status of 0–1; had no autoimmune disease; and received therapy for up to 2 years or until confirmed progression, unacceptable toxicity, or investigator decision[155]. Overall response rate (confirmed and unconfirmed) was 23% ($n = 5$), stable disease as a best response occurred in 18% ($n = 4$), and progressive disease in 59% ($n = 13$); one patient did not have response assessed at the time of analysis[155]. In the other study[155], 39 patients with PD-L1-positive gastric cancers who had received at least one previous line of therapy were treated with pembrolizumab 10 mg/kg every 2 weeks for up to 24 months. In total, 22% achieved an objective response confirmed by independent assessment[155]. PD-L1 expression level was found to be associated with the overall response rate (1-sided $P = 0.10$). The 6-month progression-free survival rate was 24%, and the 6-month overall survival rate was 69%[155].

Preclinical data showed that the dual blockade of PD-1 and cytotoxic-T-lymphocyte-associated antigen 4 (CTLA-4) was associated with increased cytokine release and increased proliferation of CD8+ and CD4+ T cells when compared with blockade of either receptor individually[156]. Furthermore, dual blockade combined with tumour vaccine has been shown to effectively restore the ability of T-cells to eradicate tumours[157]. An ongoing phase Ib/II trial is investigating the activity of nivolumab (another anti-PD-1 antibody) alone or

combined with ipilimumab (an anti-CTLA-4 antibody) in patients with advanced-stage solid tumours, including some with meta static gastric cancer[158]. Despite the preliminary nature of these data, several multinational phase III studies are assessing the value of PD-1 and PD-L1 targeting agents in different lines of treatment for patients with advanced-stage gastroesophageal cancer. The optimal selection of patients with gastroesophageal cancer for immunotherapy remains to be determined.

Previous studies assessed different cancer vaccines and adoptive immunotherapies for advanced-stage gastric cancers, but none of these approaches reached the level of prospective randomized trials sufficiently powered to prove their efficacy. It will be exciting to see if novel technologies based on processing and re-infusion of tumour-infiltrating lymphocytes will change the research landscape in oesophageal and gastric cancers, as proof-of-concept studies for epithelial cancers with chimeric antigen receptor (CAR) T-cells engineered to target HER2 or other tumour antigens have been published[159–161]. In 2015, an international consortium published data on gastric cancer immune signatures and their geographical variation[162]. Investigation of >1,600 gastric cancers revealed that tumour immune signatures differ significantly between cancers from Asian and non-Asian patients. Gastric cancers from non-Asian patients were associated with enrichment of tumour-infiltrating T-cells as well as T-cell gene-expression signatures, including those associated with CTLA-4 signalling[162]. Exploratory analysis suggests that these differences in antitumour immunity might contribute to geographical differences in clinical outcome. The design of future gastric cancer trials, particularly in immuno-oncology, should consider differences in antitumour immunity in patients from different geographi cal localities, as these might affect treatment response and clinical outcomes[162].

Conclusions

Gastroesophageal cancers are a major cause of cancer-related morbidity and mortality worldwide. Despite major treatment advances, the prognosis remains poor for both localized and advanced disease stages, owing to the molecular complexity and heterogeneity of gastroesophageal cancer. In recent years, the characterization of oesophageal and gastric cancer into subtypes based on genotype and histology has evolved, and provides a roadmap for the development of new drugs and combinations, for patient stratification and, therefore, for trials of targeted therapies. Targeting of HER2 is effective in patients with tumour expression of this RTK, in whom trastuzumab prolongs survival in combination with chemotherapy. The novel anti-HER2 antibody pertuzumab and the antibody–drug-conjugate T-DM1 are currently under investigation in this setting. Anti-HER2 treatment is now also being studied in the perioperative setting to increase response rates, and ultimately survival, in patients undergoing curative surgery. Optimal patient selection by testing of HER2 positivity remains challenging, however. The roles of EGFR, MET and FGFR as targets for drug therapy remain to be elucidated. To date, randomized studies in poorly selected patient populations with respective inhibitors of these RTKs have been unsuccessful in demonstrating a clinical benefit. As for anti-HER therapy, optimal patient selection remains challenging. Targeting angiogenesis is an emerging concept in the management of advanced-stage gastroesophageal cancer, and ramucirumab has been associated with prolonged survival in the second-line, either as a monotherapy or in combination with paclitaxel.

Immune-checkpoint inhibition and inhibition of cancer stemness are other emerging directions for the medical treatment of gastric cancer. Large-scale international studies are ongoing and more results will be reported soon. Hopefully, further insights into the molecular characteristics of gastric cancer will stimulate the role of biomarker-driven targeted therapy in gastroesophageal cancer, in both the metastatic and perioperative settings.

Biographies

Florian Lordick is Professor of Oncology and Director of the University Cancer Centre Leipzig (UCCL) at the University Hospital Leipzig in Germany. He is a member of the board of directors of the German Cancer Society and Secretary of the European Organisation for Research and Treatment of Cancer (EORTC) Gastrointestinal Tract Cancer Group. In 2015, he was elected as incoming President of the International Gastric Cancer Association Congress, to be held in Prague, Czech Republic, in May 2019. Professor Lordick's scientific focus is on clinical and translational research in gastrointestinal cancer, the optimization of multimodal care, new drug development, molecular imaging, and response prediction. He has received research support and grants from the German Cancer Society (DKG), from the German Ministry of Education and Research (BMBF), and from the Deutsche Forschungsgemeinschaft (DFG).

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Key points

- Oesophageal and gastric cancers are aggressive tumours that result in more than 1 million deaths annually worldwide
- Oesophageal and gastric cancers harbour a high number of genetic and molecular alterations, some of which contribute to an aggressiveness phenotype resulting in early development of drug resistance
- A new molecular classification of gastric cancer into four subtypes on the basis of genotypic, epigenetic and proteomic characteristics has been developed
- HER2-targeting with trastuzumab remains an important strategy in patients with HER2-positive, advanced-stage gastric cancer; however, novel anti-HER2 targeted drugs are being explored in the advanced-stage and perioperative treatment settings
- Antiangiogenic treatment with ramucirumab has proved effective in a biologically unselected patient population with disease progression after first-line therapy
- Treatments that target cancer stemness and immune-based therapies are two evolving concepts in the management of advanced-stage gastroesophageal cancer

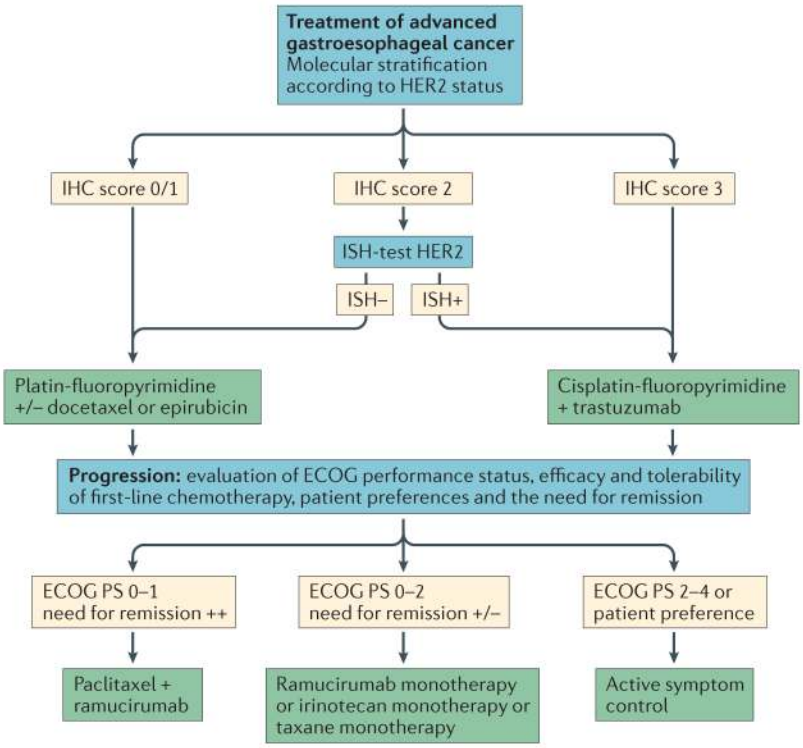

Figure 1. Proposed treatment algorithm for advanced gastroesophageal cancer based on publish recommendations[7,13]

ECOG, Eastern Cooperative Oncology Group; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization. Adapted with permission © Lordick, F. *Nat. Rev. Clin. Oncol.* **12**, 7–8 (2015).

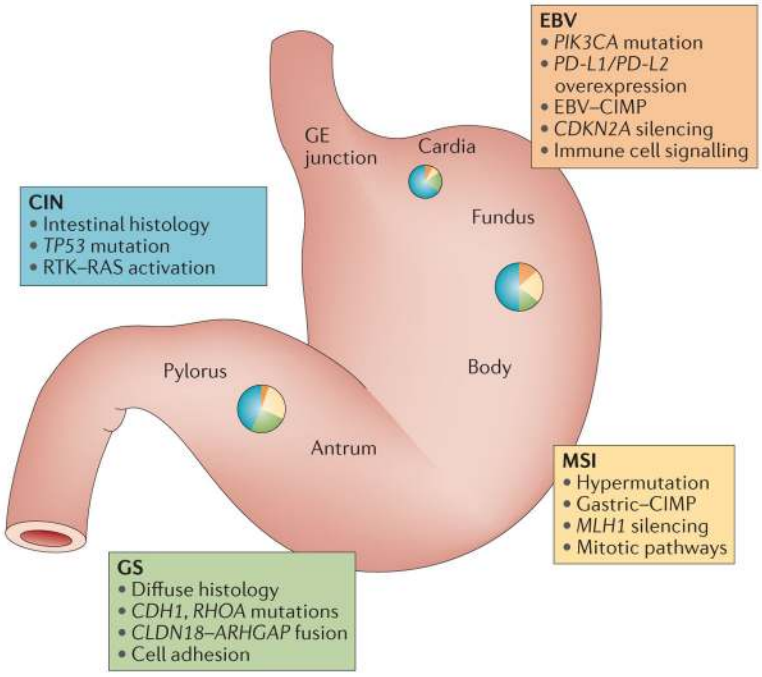

Figure 2. Key features of gastric cancer subtypes according to The Cancer Genome Atlas (TCGA)[37]

This schematic lists some of the salient features associated with each of the four molecular subtypes of gastric cancer identified by the TCGA. Distribution of molecular subtypes in tumours obtained from distinct regions of the stomach is represented in inset charts. CIMP, CpG island methylator phenotype; CIN, chromosomal instability; GE, gastroesophageal; GS, genomically stable; MSI, microsatellite instability. Reproduced with permission © Bass, A. J. *et al. Nature* **513**, 202–209 (2014)[37].

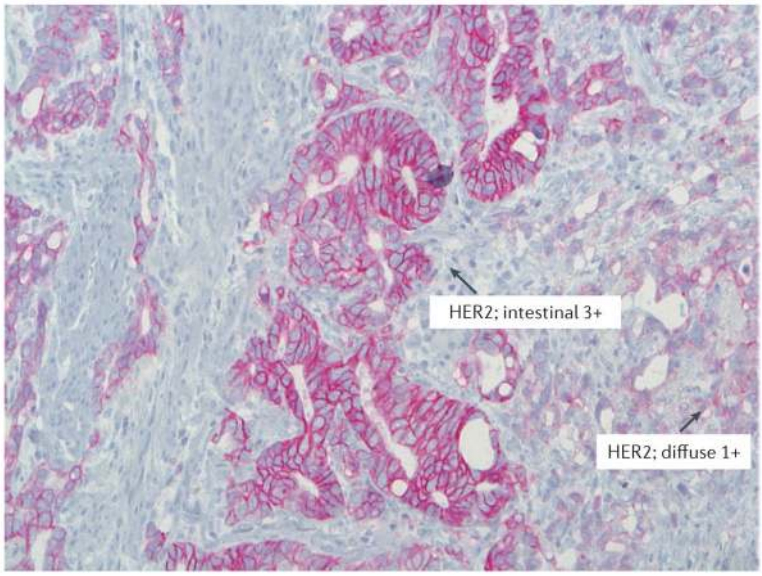

Figure 3. Biopsy of a gastric cancer

Gastric cancer biopsy with a mixed histological type showing intestinal areas with strong HER2 immunoreactivity adjacent to diffuse areas with weak staining for HER2.

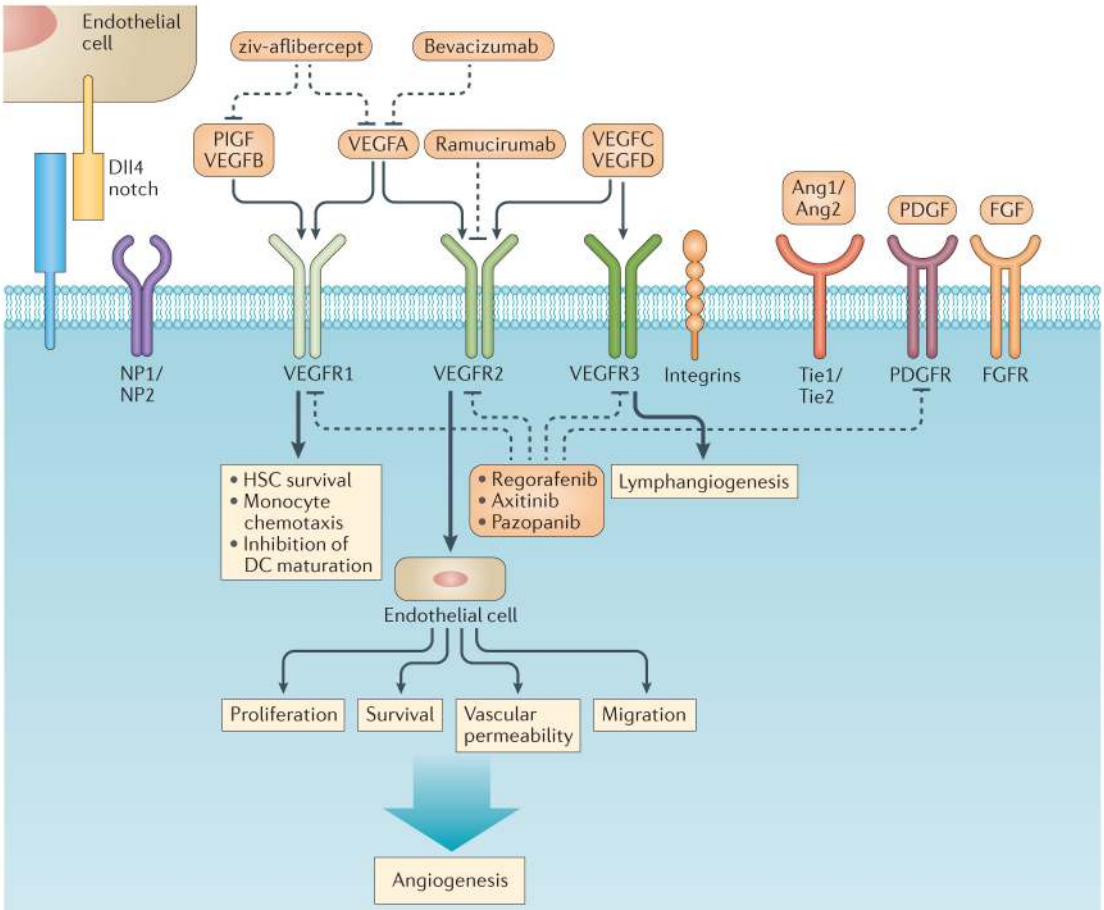

Figure 4. Angiogenic signalling network and inhibition by antiangiogenic drugs

Binding of VEGFR2 results in intracellular phosphorylation and activation of multiple downstream pathways, including PLCγ, MAPK, PI3K, AKT, and SRC. Proangiogenic signals and VEGFR activity are modulated by several receptors, including integrins, FGFR, PDGFR, Notch, and TIE2. Activation of VEGFR1 leads to downstream effects on haematopoietic stem cells (HSCs), dendritic cell (DC) maturation, and chemotaxis, whereas VEGFR3 signalling promotes lymphangiogenesis. General drug targets are illustrated for aflibercept, bevacizumab, ramucirumab, and multiple receptor tyrosine kinase inhibitors. Adapted with permission © Clarke, J. M. & Hurwitz, H. I. *Expert Opin. Biol. Ther.* **13**, 1187–1196 (2013)[123].

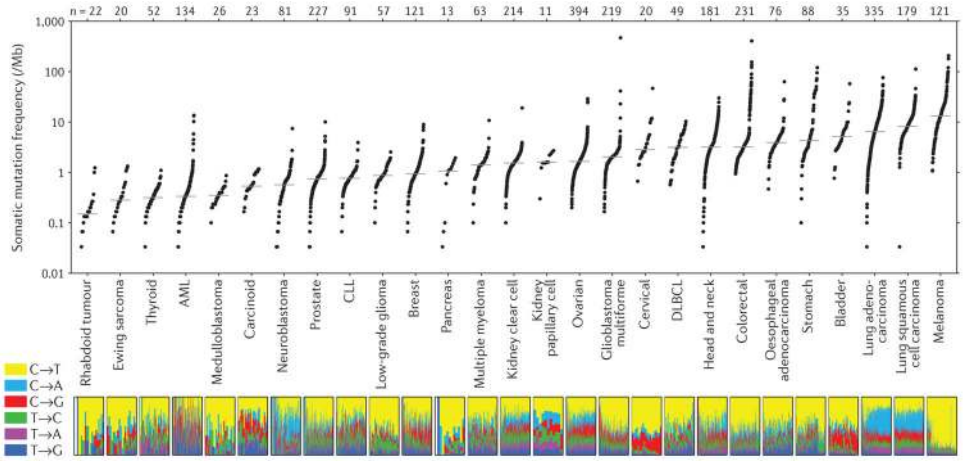

Figure 5. Mutational heterogeneity in oesophageal and gastric cancer

Mutation frequencies observed in exomes from 3,083 tumour–normal pairs are shown [L153]. Each dot corresponds to a tumour–normal pair, with vertical position indicating the total frequency of somatic mutations in the exome. Tumour types are ordered by their median somatic mutation frequency, with the lowest frequencies (left) found in haematological and paediatric tumours, and the highest frequencies (right). The lowest and highest mutation frequencies vary more than 1,000–fold across different cancers and also within several tumour types. The bottom panel shows the relative proportions of the six different possible base-pair substitutions, as indicated in the legend on the left. Reproduced with permission © Lawrence, M. S. *et al. Nature* **499**, 214–218 (2013)[152].

Table 1

Results of antiangiogenic therapy trials in gastric cancer

Study, reference and no. of patients	Treatment	Overall survival (months)	QoL and symptom control
REGARD Fuchs *et al.* (2014)[11] (*n*=355)	Ramucirumab and BSC versus placebo and BSC	5.2 months versus 3.8 months (*P*=0.047)	Improved symptom control and longer stabilization of QoL (36% versus 18% of patients had improved or stable QoL after 6 weeks *)
RAINBOW Wike *et al.* (2014)[12] (*n*=665)	Ramucirumab plus paclitaxel versus placebo plus paclitaxel	9.6 months versus 7.4 months (*P*=0.017)	Not yet fully published

BSC, best supportive care; QoL, quality of life.

* Patients were classified as improved or worsened if the change from baseline was 10 points or more, on the basis of an EORTC QIQ C30 standardized 100-point scale; changes of less than 10 points were considered as stable.