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Personalised Treatment in Gastric Cancer: Myth or Reality?

Noelia Tarazona[1,2] · **Valentina Gambardella**[1,3] · **Marisol Huerta**[1] · **Susana Roselló**[1] · **Andrés Cervantes**[1]

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Abstract Despite recent diagnostic and therapeutic advances, the survival of patients with gastric cancer is still poor. The majority of patients are diagnosed with advanced disease and chemotherapy represents the only possible therapeutic approach. However, chemotherapy seems to have reached an efficacy plateau in this setting. Gastric cancer is a complex and heterogeneous disease because it emerges from multiple interactions of genetic, environmental and host factors. A better understanding of its molecular characteristics may lead to an improvement of outcomes. The recent molecular classification by The Cancer Genome Atlas project divides gastric cancer into four subtypes that could be taken into consideration in future clinical trials with targeted agents. So far trastuzumab, a monoclonal antibody addressing the HER2 receptor, is the only targeted agent approved in the first-line setting, but only in patients overexpressing HER2. Negative data have been obtained in first-line therapy when antiangiogenics, anti-EGFR or anti-MET monoclonal antibodies have been studied in randomised controlled trials. Ramucirumab, a monoclonal antibody binding to VEGFR2, is the only antiangiogenic agent currently recommended in patients progressing after first-line treatment. In this review, we discuss whether personalised therapy may have a role in gastric cancer.

Keywords Gastric cancer · Targeted therapy · Trastuzumab · Molecular classification · Biomarkers · Patient selection · Clinical trial

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Noelia Tarazona and Valentina Gambardella contributed equally to this work.

✉ Andrés Cervantes
andres.cervantes@uv.es

[1] Department of Medical Oncology, Biomedical Research Institute INCLIVA, University of Valencia, Valencia, Spain

[2] Rio Hortega Contract CM15/00246, Valencia, Spain

[3] ESMO Translational Research Fellow, Valencia, Spain

Introduction

Gastric cancer (GC) is a global health issue and the third leading cause of cancer death worldwide [1]. Over one million new cases of GC are diagnosed each year and more than 70 % occur in developing countries, especially in East Asia [2]. Its high mortality is associated with both the absence of significant symptoms in the early stages and the lack of validated screening programmes in western countries. Consequently, most cases are diagnosed at an advanced stage, which is related to a poor prognosis [3, 4]. GC is a complex, heterogeneous and multifactorial disease. Chemotherapy (CT) remains the main treatment for advanced disease and median overall survival (OS) is around 12 months [5]. There is an urgent need for new treatments and strategies to improve outcomes. However, although multiple targeted agents are under investigation, so far, only trastuzumab and ramucirumab have demonstrated efficacy in advanced GC and have a regulatory approval [6••, 7••].

The Age of Chemotherapy

Prognosis of GC is mainly related to the stage of the disease at diagnosis. Radical surgery remains the only curative treatment for patients diagnosed with localised disease. Despite this, the expected 5-year survival rate is around 30 % in western

countries. [8] GC is mostly diagnosed in locally advanced or metastatic stage, causing a reduction of survival [5]. Up to now, CT alone has been the cornerstone of palliative treatment for advanced disease.

A meta-analysis of clinical studies that assessed CT in this setting demonstrated a significant benefit in OS for the group that received CT versus best supportive care only (BSC) (HR 0.37, 95 % CI 0.24–0.55) [9]. Combination with platinum derivatives plus fluoropyrimidines remains the standard of care for first-line regimens. However, there are some drugs that have currently been added because of superiority designed studies, such as docetaxel, or because they appear to be non-inferior in randomised studies, i.e. capecitabine, S-1, oxaliplatin and irinotecan. Some phase III trials showed that two oral fluoropyrimidines such as capecitabine and S-1 can replace infusional 5-FU in the first-line setting [10–12]. S-1 plus oxaliplatin (SOX) is esteemed as the standard first-line CT in Japan. A phase III study suggested that SOX was non-inferior to cisplatin plus S-1 (CS) in terms of survival. However, SOX provided a substantial advantage in safety over CS [13]. The 5-FU, leucovorin, and oxaliplatin regimen appeared to reduce the toxicity profile and may be an alternative to cisplatin regimen for the treatment of advanced GC [14]. Furthermore, the combination of epirubicin, oxaliplatin, and capecitabine (EOX) has been found to be as effective as the epirubicin/cisplatin/5-FU (ECF) regimen [15, 16]. Three randomised clinical trials, one with docetaxel versus BSC [17], another with irinotecan versus BSC and a third one with paclitaxel versus irinotecan versus BSC [18] demonstrated that second-line treatment did prolong survival in advanced GC patients with good performance status [19].

However, CT has reached a plateau of efficacy for GC with a median OS of around 12 months. Therefore, in an effort to enhance its activity, the combination of cytotoxic therapy with biological agents is a challenge that requires extensive research.

From Microscopic to Molecular Classification

Lauren classified gastric adenocarcinoma into two main subgroups, intestinal and diffused, according to different microscopic features [20]. Nowadays, the recommended WHO pathological classification for GC considers several subtypes, according to morphological features based on optical microscopy, identifying tubular, papillary, mucinous and poorly cohesive (including signet ring cell carcinoma), plus uncommon histologic variants [21]. However, this approach although commonly used in pathology reports does not have any predictive value when deciding management of localised or advanced GC patients. The development of taxonomic studies paying attention to molecular abnormalities observed in extended international series of GC patients is of paramount importance. Initial observations identified 22 recurrent genomic alterations in GC. Among them, already known deregulated pattern, such as ERBB2 and FGFR2, were recognised, as well as never described amplification in KLF5 and GATA6. According to this analysis, FGFR2, KRAS, ERBB2, EGFR and MET were observed to be frequently amplified in a mutually exclusive manner [22••]. Another critical contribution led to the comprehensive genomic analysis of GC developed by The Cancer Genome Atlas Research Network (TGCA) [23••]. This international cooperation identified an extensive panel of molecular abnormalities focusing on gene mutations, somatic copy number alterations, structural variants, epigenetic and transcriptional changes involving mRNAs and noncoding RNAs, as well as proteomic changes. The main aim of this collaborative effort was to define a robust molecular classification, recognising that GC is a very heterogeneous disease. The observed alterations have been matched with specific GC subtypes, permitting the identification of four major subgroups: EBV-infected, MSI tumours, genomically stable and chromosomally unstable tumours (CIN). The first group was significantly enriched for high EBV burden and showed extensive DNA promoter hypermethylation. The second group was enriched for MSI and characterised by elevated mutation rates and hypermethylation, including hypermethylation at the MLH1 promoter. Differences between them consisted of their specific spectra of mutations and gene expression. All EBV-positive tumours assayed displayed CDKN2A promoter hypermethylation but lacked the MLH1 hypermethylation characteristic of MSI-associated CIMP. The third group, defined by the lack of molecular alterations and named as genomically stable, correlates very well with the Lauren diffuse subtype. The fourth group included chromosomally unstable tumours and could be recognised by the presence of extensive somatic copy-number aberrations. Although this important molecular classification is so far lacking predictive utility, some cases with emerging genomic targets and potential therapeutic interest, such as PIK3CA, are found specifically in some groups. A strong presence of PIK3CA mutation was observed in the EBV group followed by the MSI group, but not in genomically stable or in the chromosomally stable groups. Furthermore, EBV-positive tumours had frequent ARID1A (55 %) and BCOR (23 %) mutations and only rare TP53 mutations while in hypermutated tumours, several mutated genes, including TP53, KRAS, ARID1A, PIK3CA, ERBB3, PTEN and HLA-B were identified. Among non-hypermutated tumours, several mutated genes were observed such as TP53, ARID1A, KRAS, PIK3CA and RNF43, but also genes involved in the b-catenin and TGF-b pathway. CDH1 somatic mutations were enriched in the genomically stable or diffuse subtype.

In particular, to try to identify genomic alteration in known signalling pathways, the analysis was focused on known candidate therapeutic pathways such as receptor tyrosine kinases (RTKs) and RAS and PI3-kinase signalling. According to

these criteria, EBV-positive tumours emerged to express PIK3CA mutations and recurrent JAK2 and ERBB2 amplifications. Mutations in PIK3CA, ERBB3, ERBB2 and EGFR, with many mutations at 'hotspot' sites were underlined also in MSI tumours. In CIN tumours, genomic amplifications of RTKs, many of which are amenable to blockade by therapeutics in current use or in development, were detected. Recurrent amplification of the gene encoding ligand VEGFA was notable given the gastric cancer activity of the vascular endothelial growth factor receptor 2 (VEGFR2) targeting antibody. The strength of IL-12-mediated signalling signatures in EBV-positive tumours suggests a robust immune cell presence. When coupled with evidence of PD-L1/2 overexpression, this finding adds rationale for testing immune checkpoint inhibitors in EBV-positive gastric cancer (Fig. 1).

In a further step, The Asian Cancer Research Group (ACRG) performed a similar analysis in more than 200 GCs, to derive a new classification model aiming at the description of molecular subtypes linked to different prognosis [24••]. Two different GC groups, microsatellite instable (MSI) and microsatellite stable (MSS) tumours, were firstly identified. The MSS group was further divided into two subtypes: MSS/EMT (with epithelial-mesenchymal transition features), defined by a lower number of mutations, and the MSS/epithelial. This last group was also divided into TP53 mutant and TP53 wild type. TP53 non mutant tumours had a high prevalence of APC, ARID1A, KRAS, PIK3CA and SMAD4 mutations. When these molecular features were correlated with clinical characteristics, it was observed that MSS/EMT tumours occurred at younger age and correlates with Lauren diffused type. On the other hand, the MSI subtype was frequently located in the antrum, more than half of the patients had an intestinal histology and early stage predominated at diagnosis (I/II). EBV infection was mainly seen in the MSS/TP53 mutant group. When a survival analysis was performed, there were substantial differences among the four groups. In particular, MSI subtype had the best prognosis, followed by MSS/TP53 mutant and MSS/TP53 wild type, while the MSS/EMT subtype was related to the worst survival. When the first site of relapse was investigated, it was also revealed that peritoneal metastasis were more frequent in patients diagnosed with MSS/EMT group, while a high percentage of liver limited metastasis was detected in patients belonging to the MSI subgroup. According to these data, this model moreover establishes a prognostic stratification of GC.

Targeted Therapy for Advanced Gastric Cancer: the Future

The identification of molecular features of GC led to the discovery of specific intracellular pathways and driver genes that contribute to carcinogenesis [23••]. As in other solid tumours, the use of targeted agents that block these signalling pathways

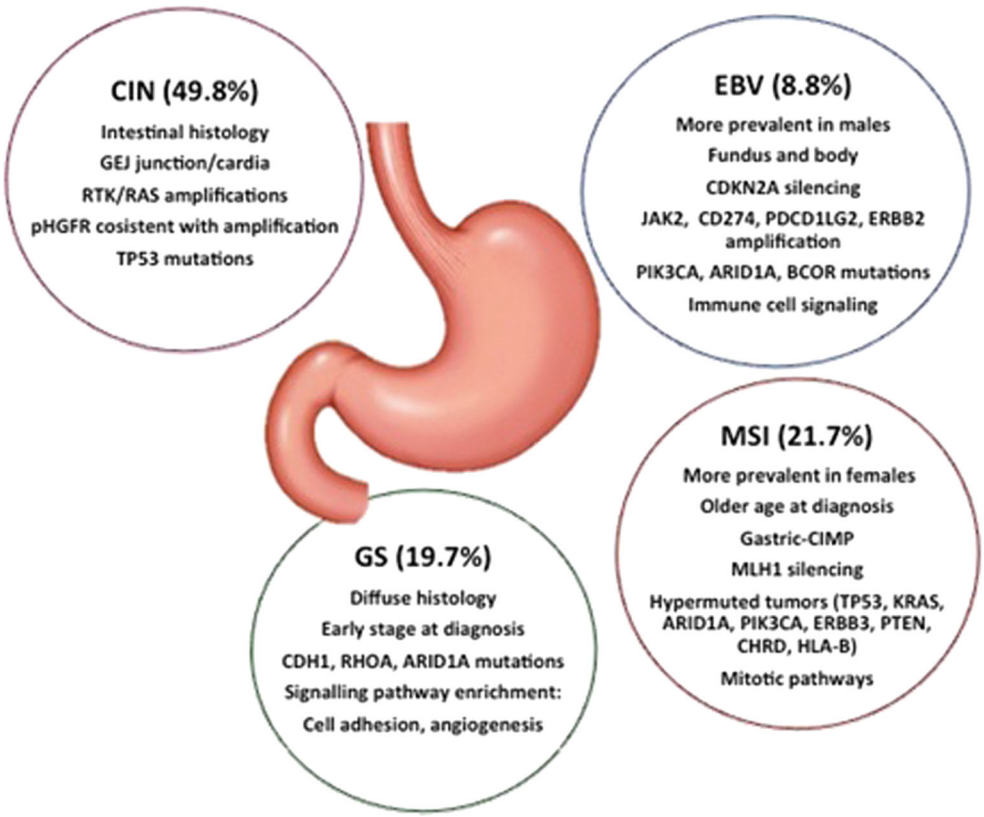

Fig. 1 Key features of gastric cancer proposed by the TCGA. *CIMP*: CpG island methylator phenotype; *EBV*: Epstein-Barr virus; *MSI*: microsatellite instability; *GS*: Genomically stable; *CIN*: chromosomal instability. Adapted with permission from Macmillan Publishers Ltd: The Cancer Genome Atlas Research Network. Comprehensive molecular characterisation of gastric adenocarcinoma. Nature 2014; 513:202–9. ©2014

has recently emerged as a strategy for the treatment of advanced GC. Targeted agents may be used either alone or in combination with cytostatic drugs. Up to now, just trastuzumab and ramucirumab have been shown to significantly improve survival in advanced GC patients [6••, 7••].

Overexpression of *epidermal growth factor receptor (EGFR)* and *Her-2* has been identified in GC activating the phosphatidylinositol 3-kinase (PI3K)/Akt and Ras/MEK signalling pathway [25]. Thus, agents that target these receptors are expected to improve efficacy of GC treatments. HER2 amplification or overexpression is detectable in 7–34 % of gastric cancer and seems to be related to poor outcomes and aggressive disease [26]. Trastuzumab, a monoclonal antibody against HER-2 receptor, was the first targeted agent approved by FDA in GC patients. A phase III, multicentre, trial (ToGA) enrolled not pretreated HER2-positive GC patients to receive cisplatin and 5-FU with or without trastuzumab [6••]. Median OS of 13.8 months versus 11.1 months was observed among patients enrolled in the experimental arm. Median progression-free survival (PFS) was 6.7 months in the trastuzumab plus CT group compared with 5.5 months in the control group. Tumour response rate, time to progression and duration of response were significantly improved in the experimental group compared with the CT alone group. By analysing subgroup characteristics, it emerged that OS was longer in tumours with high expression (immunohistochemistry 2+ and FISH positive or immunohistochemistry +3) of HER2 protein, rather than in those with low expression. Tests to identify HER2-amplified tumours are mandatory in all patients with GC, when diagnosed at advanced stage. Trastuzumab should be always used in association with platinum-based CT as first-line treatment in patients with HER2-positive metastatic GC. Pertuzumab, a recombinant humanised monoclonal antibody that inhibits HER2 homodimerisation and heterodimerisation, has been shown to improve on Trastuzumab effects in HER2-amplified breast cancer [27]. The JACOB trial is currently accruing patients to evaluate the efficacy and safety of pertuzumab, combined with trastuzumab and cisplatin plus 5-FU/capecitabine for metastatic GC or gastroesophageal junction (GEJ) cancer [28].

However, other tyrosine kinase inhibitors (TKIs) blocking the HER2-activated receptors such as lapatinib did not show that benefit. Lapatinib is blocking both EGFR and HER-2 and was assessed in the phase III TRIO-0137/(LOGIC) trial comparing the efficacy of capecitabine and oxaliplatin with or without lapatinib in HER2-amplified GC patients. Lapatinib did not significantly improve median OS. However, a subgroup analysis indicated that patients younger than 60 years and of Asian origin benefited from the addition of lapatinib [29••]. A phase III trial, TyTAN, determined that lapatinib combined with paclitaxel as a second-line regimen in patients with HER2-amplified advanced GC had superior antitumour activity with single-agent paclitaxel, but no survival prolongation was achieved [30••]. However, in Asian patients, this combination showed increase in survival compared to paclitaxel alone. Although additional prospective studies of lapatinib in HER2-amplified Asian patients could be required, its general use cannot be currently recommended.

The blockage of EGFR or HER1 has also been investigated in GC. Cetuximab, a chimeric IgG1 monoclonal antibody, combined with capecitabine and cisplatin (EXPAND trial) and panitumumab, a fully humanised monoclonal antibody, combined with EOX (REAL-3) as a first-line regimen have not demonstrated improvements in survival among advanced GC patients [31••, 32••]. Likewise, several TKIs of EGFR have been tested. Gefinitib, the first TKI to treat cancer, was well tolerated but of limited efficacy in patients with recurrent or metastatic oesophageal or GEJ cancer due to rare EGFR mutations [33]. Erlotinib (SWOW 0127 trial) is active in patients with GEJ adenocarcinomas but appears inactive in GC [34]. Intratumoral EGFR, transforming growth factor-alpha or phosphorylated Akt kinase expression were not predictive of clinical outcome. Neither somatic mutations of the EGFR exons 18, 19, or 21 were detected nor was there gross amplification of EGFR.

Vascular endothelial growth factor (VEGF) is one of the most important inducer of tumour angiogenesis. VEGF expression is commonly high in GC tissues and is related to the invasiveness, clinical stage and prognosis of GC [35]. Anti-VEGF antibodies and VEGF inhibitors are expected to block angiogenesis and downstream signalling. In the phase III trial, AVAGAST, bevacizumab adding to capecitabine in the first-line treatment of advanced GC did not extend OS but was associated with significant increases in PFS and overall response rate [36]. A subgroup analysis demonstrated longer OS for non-Asian patients and the efficacy of bevacizumab seems to be related to the baseline expression of VEGF-A and Neuropilin-1 [37••]. Ramucirumab, a completely humanised monoclonal antibody against VEGFR2, demonstrated either alone or in combination with paclitaxel (RAINBOW Trial) survival and disease control rate benefit as second-line regimen for non-Asian GC patients [7••, 38••]. The absence of survival benefit in the AVAGAST and RAINBOW among the Asian patient subset could be justified by the better OS recorded in Asian patients regardless of treatment. Another antiangiogenic drug, Apatinib, has been tested in a phase III clinical trial. Apatinib is a high selective small-molecule tyrosine kinase inhibitor that strongly inhibits VEGFR2. In a phase III double blinded, placebo-controlled Asian trial, GC patients who progressed to at least two lines of CT were randomised to receive apatinib versus placebo. Median OS was significantly improved in the apatinib group compared with placebo (6.5 months versus 4.7 months; HR 0.709); PFS was also prolonged: 2.6 versus 1.8 months [39••]. Basing on the results of the last studies, whether it is better

to target the VEGFR2 by a tyrosine kinase inhibitor or a monoclonal antibody is a matter of investigation [39••, 40].

Others agents that have currently been evaluated in phases II trials include sunitinib and sorafenib. Sunitinib, an oral multitargeted TKI, in combination with docetaxel improved the objective remission rate compared to docetaxel alone (41.1 vs 14.3 %, respectively) [41]. Sorafenib, another multikinase TKI, combined with docetaxel and cisplatin was associated with 5.8 median PFS and a 13.6-month median OS [42]. A trial combining sorafenib with capecitabine and oxaliplatin is ongoing. These findings require further investigation in randomised controlled phase III trials.

Overexpression of hepatocyte growth factor and *MET* has been reported in 73–88 % and 26–82 % of advanced GC patients, respectively. Moreover, overexpression of MET has been associated with poor prognosis in this setting [43]. A phase II study showed that a combination regimen of rilotumumab, a c-Met inhibitor, with ECX for the treatment of advanced gastric and GEJ cancer provided a survival benefit [44]. Based on these, two randomised trials were conducted, RILOMET-1. Regrettably, the RILOMET-1 study, the rilotumumab plus ECX regimen as first-line therapy for MET-positive GC patients has been reported as negative [45••]. Onartuzumab, an *Escherichia coli*-derived, humanised, monovalent monoclonal antibody against Met, when administered as a single agent or in combination with bevacizumab in patients with advanced solid malignancies, showed that onartuzumab was generally well tolerated and that it showed antitumour activity in GC [46]. Although the results of these studies are not published, onartuzumab has been tested in phase II and III studies in combination with FOLFOX to treat patients with metastatic HER2-negative solid tumours [47]. Foretinib is an active TKI of MET, ALK and VEGFR2, in under further research in GC [48]. Another experimental MET TKI, AMG377, is also very active in advanced GC as shown in early clinical trials [49]. It could be the case that TKIs inhibiting MET are more potent in the clinic than monoclonal antibodies binding to the MET receptor or its ligands.

Fibroblast growth factor (FGF) pathway has been recognised to be a promising target, and clinical trials are testing its inhibition. FGFR is implicated in tumorigenesis and chemoresistance [50]. Several small-molecule FGFR inhibitors are currently in clinical development. AZD4547 is a novel selective small-molecule inhibitor of FGFR with potent antitumour activity against FGFR-deregulated tumours in preclinical models. Analysis of two phase II studies of AZD4547 was presented at the 2015 ASCO congress. One of them using AZD4547 monotherapy in FGFR2-amplified GC patients who had progressed after one or more lines of CT resulted positive [51]. AZD4547 demonstrated high activity in FGFR2-amplified GC patients. Copy number variation using droplet digital PCR in tumour tissue and plasma identified all GC responders. Digital imaging demonstrated high levels of homogenous FGFR2 amplification in responding GC patients. However, the SHINE study of AZD4547 monotherapy versus paclitaxel for patients with FGFR2 polysomy or gene amplification was reported to be negative [52]. We could justify this discrepancy because the optimal selection patients did in the former clinical trial.

The *PI3K/AKT/mTOR pathway* is frequently activated in GC, with overexpression of PI3KCA and phosphorylation of AKT in 35–80 % and in 40–82 % of GC cases, respectively [53]. A phase III study, GRANITE-1, compared everolimus versus BSC in terms of efficacy and safety in advanced GC that progressed after one or two lines of previous CT. Everolimus did not significantly improve OS in this setting [54••].

The abnormal *expression of matrix metalloproteinase* (MMPs) is associated with the progression and poor prognosis of GC [55]. The MMP inhibitor marimastat exhibits antitumour activity in GC [56]. A summary of all phase three studies with targeted agents investigated in any metastatic disease setting is provided in Table 1.

In GC, a particular pattern of mutation was recently detected while studying signatures of mutational processes in human cancers [57]. This pattern in breast and ovarian cancer coincides with BRCA1 and BRCA2 mutations, suggesting that some GCs might harbour BRCA1 and BRCA2 mutations or even mutations, called as signature 3, leading to similar effects. Most of these mutations in GC were heterozygous and were found in cases with a very high prevalence of small indels and base substitutions, due to defective MSI and acting as passenger mutations. This molecular signature suggests a possible therapeutic approach for PARP inhibitors in GC [58••]. Based upon the understanding of deregulated DNA damage response, a phase II randomised placebo-controlled trial was conducted to investigate olaparib plus paclitaxel in second-line GC patients. An analysis identifying ataxia telangiectasia gene (ATM) expression was also performed. PARP inhibitors could cause synthetic lethality in ATM low expression. The primary endpoint, PFS, was not met, probably because of the difficult evaluation of patients with non-measurable disease, such as peritoneal carcinomatosis. Nevertheless, olaparib plus paclitaxel significantly improved OS versus placebo/paclitaxel in both the overall (HR, 0.56; OS, 13.1 versus 8.3 months) and the ATM low population (HR, 0.35; median OS, not reached versus 8.2 months) [59••, 60].

Discussion

The treatment of advanced GC is essentially palliative. Although it has been considered as a chemosensitive tumour for many years, no significant progress in its management has resulted within the last two decades. Most responses to CT are partial and have short duration. In metastatic patients, median

Table 1 Phase III clinical trials that used targeted agents for the treatment of advanced GC

Trial	Agent	Chemotherapy	Line Treatment	OS (experimental vs control group)	PFS (experimental vs control group)
EGFR/ERB2 pathway					
ToGA [6••]	Traztuzumab	X(FU)P	First-line	mOS 13.8 m vs 11.1 *P* = .0046	mOS 6.7 m vs 5.5 m *P* = 0 · 0002
EXPAND [31••]	Cetuximab	XP	First-line	mOS 9.4 m vs 10.7 m *P* = .95	mPFS 4.4 m vs 5.6 m *P* = .32
REAL3 [32••]	Panitumumab	EOX	First-line	mOS 8.8 m vs 11.3 m *P* = .013	mPFS 6.0 m vs 7.4 m *P* = .068
TRIO-013/ LOGIC [29••]	Lapatinib	XELOX	First-line	mOS 12.2 m vs 10.5 m *P* = .0381	mPFS 6.0 m vs 5.4 m *P* = 0 · 381
TyTAN [30••]	Lapatinib	Paclitaxel	Second-line	mOS 11.0 m vs 8.9 m *P* = .1044	mPFS 5.4 m vs 4.4 m *P* = .2441
VEGF pathway					
AVAGAST [36]	Bevacizumab	X(FU)P	First-line	mOS 12.1 m vs 10.1 m *P* = .1002	mPFS 6.7 m vs 5.3 m *P* = .0037
REGARD [38••]	Ramucirumab	BSC	Second-line	mOS 5.2 m vs 3.8 m *P* = .0047	mPFS 2.1 m vs1.39 m *P* = unknown
RAINBOW [7••]	Ramucirumab	Paclitaxel	Second-line	mOS 9.6 m vs 7.4 m *P* = .017	mPFS 4.4 m vs 2.9 m *P* < .0001
NCT01512745 [39••]	Apatinib	placebo	Third-line	mOS 6.9 m vs 4.7 *P* = .0149	mPFS 2.6 vs 1.8 *P* < *.001*
PI3K/Akt/mTOR pathway					
GRANITE-1 [54••]	Everolimus	placebo	After one or two lines	mOS 5.4 m vs 4.3 m *P* = .124	mPFS 1.7 m vs 1.4 m *P* < .001
MET pathway					
RILOMET1[45••]	Rilotumumab	ECX	First-line	mOS 9.6 m vs 11.5 m *P* = .021	mPFS 5.7 m vs 5.7 m *P* = .025
MetGastric [47]	Onartuzumab	mFOLFOX6	First-line	No study results posted	No study results posted

X Capecitabine, *5FU* 5-fluorouracil, *P* platinum, *EOX* epirubicin/oxaliplatin/capecitabine, *XELOX* capecitabine/oxaliplatin, *BSC* best supportive care, *ECX* epirubicin/cisplatin/capecitabine

survival achieved with CT is 6–11 and survival at 2 years is exceptionally >10 % [61, 62]. As a result of advances in the field of tumour biology, attention has been focused on the new modality of molecular targeted therapy for advanced cancer. Although an increasing number of clinical studies have explored the effect of targeted treatment alone or in combination with CT, its application in GC remains in its infancy compared with its successful use in colon, lung, and breast cancers. Precision medicine for GC continues to face important challenges. Many phase II clinical trials have been performed. However, randomised phase III clinical trials are scarce. Despite the great interest derived from the identification of alteration such as EGFR and HER2 amplification and PI3KCA mutations, in several clinical trials, the benefit obtained by using targeted agents against these was not observed. The use of anti EGFR monoclonal antibodies, cetuximab and panitumumab did not lead to clinical benefit [31••, 32••]. One of the reasons, which could have caused this result, is the choice of capecitabine and cisplatin backbone CT. In a clinical trial, enrolling KRAS wild-type colorectal cancer patients, cetuximab plus first-line CT regimens based on an oral fluoropyrimidine, or bolus fluorouracil, does not provide additional benefit in patients compared with CT alone [63]. The reason is unclear and might be attributable to a negative pharmacokinetic interaction between capecitabine and cetuximab or increased incidence of toxic effects associated with this treatment combination compared with cetuximab and infusional fluorouracil. In both trials, EGFR overexpression was not preselected as inclusion criteria. Because of the increase of gastrointestinal toxicity due to the use of panitumumab, in the REAL 3 trial, the schedule of backbone CT used in the study protocol was partially modified, and this variation could justify the detrimental trend observed. Another possible reason of the failure of this strategy

was supposed to be the not completely correct patient selection. Data from single-group studies in this setting suggest that EGFR expression, EGFR gene copy number and expression of other EGFR ligands (epiregulin and amphiregulin) or downstream components of the EGFR-signalling pathway might be candidate biomarkers for EGFR-antibody efficacy [64]. In the EXPAND trial, EGFR tumour expression was generally low, and a subgroup analysis suggested that the EGFR immunohistochemistry score was not associated with survival in either treatment group [31••]. Furthermore, as in GC, the expected rate of KRAS mutation was low, and KRAS was not used as a predictive biomarker.

Despite the advantage in adding an anti-HER2 treatment in HER2-positive GC, the use of lapatinib did not show any benefit in Caucasian both in first-line and in second-line treatment [29••, 30••]. Many possible causes could have led to these results such as the superior efficacy of trastuzumab against HER2-positive tumours and the lack of compliance of patients because of the presence of gastrointestinal toxicities related to lapatinib. Another relevant aspect, which could be related with the disappointing results, is the pharmacokinetics of lapatinib that could have been deeply modified because of previous gastric surgery, as suggested when lapatinib was added to paclitaxel as second-line treatment. Moreover, unlike the ToGA trial, a relevant difference was observed above different ethnic groups, suggesting that Asian patients could benefit from this treatment, even not reaching statistical significance. According with data extrapolated in ToGA trial, in second-line setting, adding lapatinib to paclitaxel could be meaningful in IHC 3+ patients but not in IHC 0/1 [6••]. When everolimus was tested versus BSC in pretreated patients in a no selected population, no clinical benefit was underlined [54••]. The lack of significant benefit may be partially attributable to the treatment after progression received by patients randomised in the placebo arm.

The absence of predictive biomarkers is making difficult the application of personalised or precision medicine to GC patients. In many trials with targeted agents, the design has not taken into account an appropriate selection process, mimicking traditional types of designs useful in the era of cytotoxic CT. When patients are selected without a precise molecular feature, which makes their tumours more sensitive to a very specific therapeutic intervention, the possibility of getting successful data is very limited. Targeted agents are not to be used for all comers. The example of trastuzumab, being studied only in HER2-amplified tumours, with a validated, feasible and available test that could be performed anywhere, is indicating us the way to proceed. If HER2 inhibitors were applied to all patients, without such a strong predictive biomarker, they would have failed in impacting survival in a general or non-selected population. Having many negative trials with targeted drugs in GS is a challenge for a better trial design, in which potential targeted molecular alterations should be systematically investigated to enrich our patient samples in trials and to eventually enhance the probability of having a successful outcome. MET is a potential driver in GC, and therefore, MET antibodies and small TKIs were tested in clinical trials without success in phase III randomised studies, despite a clear evidence of extensive and durable responses in individual patients during earlier development [43, 44, 45••, 46, 47]. Developing predictive biomarkers at the same time that targeted drugs are studied in early clinical trials is a need for a better personalised approach.

The use of antiangiogenic drugs was supposed to be active but the first randomised trial using bevacizumab did not confirm this hypothesis [36]. Despite this evidence, the use of another antiangiogenic, ramucirumab, alone or in combination with paclitaxel as second-line treatment demonstrated a benefit in both OS and PFS [7••, 38••]. Ramucirumab, a fully humanised antibody, is a high selective inhibitor of VEGFR2. The advantage of inhibiting directly this receptor could be associated with better clinical outcomes [65]. Likewise, the absence of survival benefit in the AVAGAST and RAINBOW trial in Asian patients could be explained by this population which has longer OS compared with western population, independent of the treatment and by the pharmacogenetic differences in drug metabolism which lead to differential toxicities and survival across regions in clinical trials. In a study which examined genotype profiles in Japanese and American patients across four randomised trials in NSCLC, Gandara et al. demonstrated variability in allelic frequencies of variants in enzymes relating to paclitaxel and cisplatin metabolism which were associated with both toxicity and patient outcomes [66]. So, we should take into account the individual differences in drug metabolism and bioavailability.

Moreover, another relevant field under investigation is the role of immunotherapy in gastric cancer. In a phase I clinical trial enrolling PD-L1 positive in stroma or ≥1 % cancer cells in patients with metastatic GC, pembrolizumab was generally well tolerated and showed antitumour activity in heavily pretreated patients. Pembrolizumab is currently undergoing extensive investigation in phase I and II trials [67].

Conclusions

We have learned that GC is a very heterogeneous disease as defined by the new molecular classification. On the other hand, validated predictive biomarkers are of extreme importance to bring new developments into clinical practice. One size does not fit all. Molecular targeted agents cannot be developed as if they were cytotoxic agents. Powerful tools such as next-generation sequencing may definitely help in understanding this complex situation. Early development of predictive biomarkers, when molecular targets are blocked by specific agents, is of paramount importance. Personalised or

precision medicine means not only better and more specific drugs but also better tools to identify as different diseases that on the classical and conventional pathology approach look very much the same. This path has already been initiated for gastric cancer, although it is in its very early steps. The example of HER2-amplified tumours is outstanding in this sense, but finding out other subgroups in which a similar approach could be applied should be our goal for future development.

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Compliance with Ethical Standards

Conflict of Interest Noelia Tarazona, Valentina Gambardella, Marisol Huerta, Susana Roselló and Andrés Cervantes declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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