Current Problems in Cancer 44 (2020) 100590

Contents lists available at ScienceDirect

Current Problems in Cancer

journal homepage: www.elsevier.com/locate/cpcancer

Progress and challenges in gastroesophageal cancer

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A B S T R A C T

Gastroesophageal adenocarcinoma (GEA) is a challenging disease; most GEA patients do not live for more than a year after a diagnosis of advanced disease. Development of effective targeted therapeutics for GEA patients lags behind other cancers. Progress in molecular biology has provided subclassifications of gastroesophageal cancer which may have prognostic and predictive utility and has identified novel therapeutic targets. Heterogeneity in biomarker expression has been a challenge in new drug development, leading to negative trials of targeted therapeutics in the first and second line setting. In this review, we discuss developments in understanding GEA biology, focus on putative prognostic and predictive biomarkers and examine the results of important recent clinical trials. The role of hetergeneity in GEA outcomes is reviewed and we discuss intra- and interpatient heterogenetiy in the context of emergent data on liquid biopsy and how this might complement tissue diagnosis and determine treatment in the GEA field. Finally, we examine recent results from international trials using immune checkpoint blockade with anti-PD-1, anti-CTLA4, and anti-PD-L1 antibodies, in an effort to dissect the interaction between gastroesophageal tumour and

☆ Conflict of interest: ECS reports honororia for advisory role from BMS, Celgene and Servier outside the submitted work.

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https://doi.org/10.1016/j.currproblcancer.2020.100590

enviroment on the immune response and we reflect on how immune checkpoint blockade may impact of treatment paradigms for GEA in future.

ARTICLE INFO

Keywords: Gastroesophageal cancer; Gastric cancer; Oesophageal cancer; Chemotherapy; Targeted therapy; Immunotherapy; Molecular Profiling; Next Generation sequencing; Clinical trials

Introduction

Gastroesophageal adenocarcinoma (GEA) is still a major cause of cancer-related mortality worldwide.[1] In addition, GEA is very heterogeneous from the molecular point of view. Over the last years, several new agents have been investigated in GEA advanced disease, however, few positive results have been obtained and most patients with metastatic disease live less than 2 years. Gastric cancer (GC) has traditionally been classified into 2 major histological subtypes according to Lauren's classification: Intestinal and diffuse types. Besides the Lauren's classification, other more modern histopathological classifications have been proposed for GC. These histopathological classifications are widely used, but they will not allow us to identify which patients will benefit from a certain therapy or strategy and which ones will not. In recent years, great efforts have been made to classify GEA molecularly. Moreover, new stategies to apply the molecular classification to daily practice with affordable cost/benefit techniques have been developed.

Progress in understanding molecular biology

Molecular characterization in gastric and esophageal cancer

The Cancer Genome Atlas (TCGA) network redefines GC into 4 distinct subtypes based on mutations, gene copy-number changes, gene expression, and DNA methylation.[2] TCGA GC molecular subtypes are chromosomal instability (CIN), the most frequent group, which represents up to 50% of the samples, Epstein Barr Virus positive (EBV) 9%, microsatellite-unstable (MSI) 21% and genomically stable (GS) 20% (Table 1). Likewise, TCGA network research suggests that histological subtypes of esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC) are distinct in their molecular characteristics; ESCC shows frequent genomic amplifications of CCND1 and SOX2 and/or TP63, whereas ERBB2, VEGFA and GATA4, and GATA6 are more commonly amplified in EAC. Taken together, the gastric and esophageal TCGA conclude that ESCC resembles more squamous carcinomas of other sites like head and neck region while there is a notable molecular similarity between EACs and CIN GCs.[3] Most of the gastroesophageal junction (GEJ) samples analyzed by TCGA were also CIN.[3] These molecular similarities between EAC/GEJ and CIN GCs suggest that they could be considered a single disease entity. However, some molecular features, for example DNA hypermethylation, manifest differently in EAC/GEJ and GC, being more frequent in EAC and GEJ than in GC.[3] Although several studies have identified mutations in 10 known cancer genes as EAC drivers: *TP53, CDKN2A, SMAD4, ARID1A, ERBB2, KRAS, PIK3CA, SMARCA4, CTNNB1, and FBXW7*,[3,4] the genomic landscape of EAC appears to be more frequently dominated by structural variation (such as copy number alterations and large-scale rearrangements) rather than mutations.[4] More recently, the Oesophageal Cancer Clinical and Molecular Stratification group has published on a cohort of 551 genomically characterised EACs using the esophageal International Cancer Genome Consortium project and including detection of noncoding driver mutations and verification of therapeutic strategies in cell lines and

Table 1
Main molecular features of GC subtypes according to TCGA report.

TCGA subtype (%)	CIN (50%)	EBV (9%)	MSI (20%)	GS (20%)
Molecular features	-Marked aneuploidy -Recurrent amplifications of RTK -*VEGFA* amplification -Cell cycle mediators amplifications -No high mutation rates, BUT recurrent *TP53* mutations	-Extreme DNA hypermethylation status: *CDKN2A* silencing in 100% samples -*PIK3CA* mutations (80%) -*JAK2* and *PD-L1/PD-L2* overexpression -*ARID1A* mutations (55%)	-DNA hypermethylation status: *MLH1* silencing in 100% -Hypermutation status: *ERBB1-3* mutations and *PIK3CA* mutations (42%)	-Lack of aneuploidy, hypermethylation or elevated rates of mutation -*RHOA* mutations (15%) -*CDH1* somatic mutations (37%) -*CLDN18-ARHGAP26* fusions -*FGFR2 and VEGFA* amplifications -*ARID1A* mutations
Typical location and Correlation with *traditional* subtypes	-Mostly of tumors at the GEJ -Lauren intestinal histologic variant	-Fundus and body	-Fundus, body and antrum	-Lauren diffuse histologic variant

ARID1A, AT-rich interactive domain-containing protein 1A; CDH1, Cadherin1; CDKN2A, Cyclin-dependent kinase Inhibitor 2A; CLDN18-ARHGAP26, Claudin18-Rho GTPase activating protein 26; EAC, Esophageal adenocarcinoma; ERBB1-3, Receptor tyrosine-protein kinase erbB1-3; FGFR2, Fibroblast growth factor receptor 2; GEJ, Gastroesophageal junction; JAK2, Janus kinase 2; MLH1, MutL homolog 1; PD-L1/L2, Programmed cell death ligand L1/L2; PI3KCA, Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; RHOA, Ras homolog gene family member A; RTK, Receptor tyrosine kinases; TP53, Tumor protein 53; VEGFA, Vascular endothelial growth factor.

organoids.[5] Mutual exclusivity or co-occurrence of events within and between a number of EAC pathways was described in this study: These included *GATA* factors, Core Cell cycle genes, *TP53* regulators, and the *SWI/SNF* complex. The study also identified novel EAC copy number drivers, for example, *CCND3, AXIN1, PPM1D*, and *APC*, as well as, validated poor prognostic indicators: *SMAD4* and *GATA4*, as independent predictors of survival. Interestingly, they found that over 50% of EACs harboured sensitising events for CDK4/6 inhibitors which where confirmed in a panel of EAC cell lines and organoids.

Prognostic and predictive value of molecular GC subtypes

Although the TCGA did not show significant differences between GC molecular subgroups in terms of survival or recurrence rates, probably due to the limited follow up at the time of its analysis, some reports have suggested that EBV and MSI subtypes of GC show an improved prognosis.[6,7] MSI or defective DNA mismatch repair are associated with improved survival in patients with stage II colon cancer and are negatively prognostic for benefit from fluoropyrimidine adjuvant chemotherapy in the same patient group.[8] Mirroring colorectal cancer literature, an exploratory analysis of The United Kingdom Medical Research Council Adjuvant Gastric Infusional Chemotherapy trial, a randomized clinical trial published in 2017, showed that patients with operable gastroesophageal cancer with high MSI had higher survival rates compared with patients with gastroesophageal cancer with low MSI or microsatellite stable tumors (MSS) when treated with surgery alone. Furthermore, patients with operable gastroesophageal cancer with low MSI or MSS had higher survival compared with patients with high MSI when treated with perioperative chemotherapy plus surgery, which suggests that high MSI subgroup may not benefit from perioperative chemotherapy.[9] Analysis of the impact of MSI on survival in the CLASSIC trial showed a similar lack of benefit for adjuvant chemotherapy in MSI patients.[10] Notably, high

MSI condition is less common in patients with advanced gastric disease and it has been associated with poor response to chemotherapy in metastatic setting.[11,12]

Tumor heterogeneity in gastroesophageal cancer

GEA is well recognized as a highly heterogeneous disease between individuals. In addition to interpatient variability, intratumoral heterogeneity within the same patient at primary and metastatic sites and even within the same tumor are frequently described. Intrapatient heterogeneity includes spatial heterogeneity in different tumor areas and temporal heterogeneity at different time points across a tumor's natural history. This intratumoral heterogeneity may have significant impact on clinical outcomes, in particular for targeted therapies. For example in an FGFR inhibitor clinical trial that was guided by *FGFR2* amplification testing of the primary tumour, many patients who were FGFR amplified according to standard criteria failed to respond to FGFR inhibitor treatment.[13] Translational trial conducted by Pearson et al on this dataset[14] demonstrated that patients who did respond to the same therapy had homogenous FGFR2 amplification, whereas patients who did not respond had more heterogeneous FGFR2 amplification in the primary tumour. Likewise, Janjigian et al[12] described that HER2 discordance between FISH/IHC and NGS could be attributed to intratumor heterogeneity in regard to ERBB2 amplification. Böger et al also report intratumoral heterogeneity of EBV infection by EBER in situ *hybridization* and *PIK3CA* mutations in GC. Importantly, intratumoral PIK3CA heterogeneity within the primary tumour was also present in the corresponding lymph node metastases.[15] More recently, Pectasides et al[16] analyzed paired primary tumours and metastatic lesions from patients enrolled in the PANGEA trial (Personalized Antibodies for GEA, NCT02213289)[17] and confirmed a high level of intrapatient heterogeneity reporting a baseline discordance between primary tumor and metastasis from approximately 40% for single-nucleotide variants and insertion-deletion elements (indel), to approximately 60% for amplified genes such as *HER2, CDK4/6, EGFR*, and *KRAS*. Interestingly, these results cannot be attributed to acquired resistance or treatment effects because no systemic treatment was started prior the biopsy/analysis. Moreover, when exploring the molecular basis for variability in responses to afatinib in GEA patients, Sanchez-Vega reported that pre-existent heterogeneity of EGFR amplification may explain the patient's mixed response to the drug due to the selection during or after treatment of a tumor clone that either lacked a sensitizing amplification (EGFR amplification) or had gained a resistance amplification (MET amplification).[18]

Liquid biopsy

Tumors release components such as circulating tumor cells or circulating tumor DNA (ctDNA). Current practice in GEA molecular characterization relies upon a single biopsy or a few primary tumour biopsies obtained during an endoscopic exam. Thus, available tumor sample consist of most often a small tissue biopsy that can be easily exhausted after carrying out the standard tests for the diagnostic and precluding further screening for trial eligibility without repeating a biopsy. Liquid biopsy shows promise as a complementary method of molecular profiling and identification of predictive mutations for targeted treatments at baseline. Recent studies in a variety of cancer types have demonstrated the feasibility of the detection in blood samples of predictive biomarkers that are relevant for daily practice.[19,20] As a result selected liquid biopsy test kits in lung and colon cancer have achieved government approval for the detection of EGFR and RAS/BRAF mutations in plasma. With respect to immune biomarkers, the viability of tumor mutational burden and PD-L1 expression assessment by liquid biopsy in patients with advanced lung cancer has also been demonstrated.[21,22] In GC, small studies have shown that HER2 amplification detected in plasma correlates well with tumor HER2 amplification.[23,24] Other potential uses of liquid biopsies are dynamic monitoring of treatment response/disease progression and early detection of secondary resistance.

Table 2
Targeted therapies for advanced GEA in first-line randomized phase III clinical trials.

Target	Study name/Ref.	Selected population	Drugs	mOS or mPFS	HR
HER2	**ToGA**[25]	**GC HER2 positive**	**Chemo ± trastuzumab**	**mOS 13.8 vs 11.1 (mo)**	**0.74 (0.60-0.91)**
HER2	TRIO/LOGIC[26]	GC and EC HER2 positive	Chemo ± lapatinib	mOS 12.2 vs 10.5 (mo)	0.91 (0.73-1.12)
HER2	JACOB[27]	GC HER2 positive	Chemo + trastuzumab ± pertuzumab	mOS 17.5 vs 14.2 (mo)	0.84 (0.71-1.00)
EGFR	EXPAND[28]	GC All-comers	Chemo ± cetuximab	mPFS 4.4 vs 5.6 (mo)	1.09 (0.92-1.29)
EGFR	REAL-3[29]	GC All-comers	Chemo ± panitumumab	mOS 8.8 vs 11.3 (mo)	1.37 (1.07-1.76)
MET	RILOMET-1[30]	GC MET positive	Chemo ± rilotumumab	mOS 8.8 vs 10.7 (mo)	1.34 (1.10-1.63)
MET/HGF	METGastric[31]	GC MET positive	Chemo ± onartuzumab	mOS 11 vs 11.3 (mo)	0.82 (0.59-1.15)
VEGFR2	RAINFALL[32]	GC HER2 negative	Chemo ± ramucirumab	mPFS 5.7 vs 5.4 (mo)	0.75 (0.61-0.94)
VEGF	AVAGAST[33]	GC All-comers	Chemo ± bevacizumab	mOS 12.1 vs 10.1 (mo)	0.87 (0.73-1.03)
MMP9	GAMMA-1	GC All-comers	Chemo ± GS-5745	mOS 12.5 vs 11.8 (mo)	0.93 (0.74-1.18)

In bold, trials with positive results.
AVAGAST, Bevacizumab in Combination With Chemotherapy As First-Line Therapy in Advanced Gastric Cancer, A Randomized, Double-Blind, Placebo-Controlled Phase III Study; chemo, Chemotherapy; EC, Esophageal cancer; EGFR, Epidermal growth factor receptor; EXPAND, Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer; GC, Gastric cancer; HER2, Human epidermal growth factor receptor 2; HGF, Hepatocyte growth factor; JACOB, Pertuzumab + trastuzumab + chemotherapy for HER2-positive metastatic gastric or gastro-oesophageal junction cancer; MET, Mesenchymal epithelial transition factor; MMP9, Matrix Metalloproteinase-9; mOS, Median overall survival; mPFS, Median progression-free survival; RAINFALL, Ramucirumab with cisplatin and fluoropyrimidine as first-line therapy in patients with metastatic gastric or junctional adenocarcinoma; REAL-3, Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer; RILOMET-1, Rilotumumab plus epirubicin, cisplatin, and capecitabine as first-line therapy in advanced MET-positive gastric or gastro-oesophageal junction cancer; ToGA, Trastuzumab for Gastric Cancer; TRIO/LOGIC, Lapatinib in combination with capecitabine plus oxalipatin in HER2 advanced or metastatic Gastric, Esophageal or Gastroesophageal adenocarcinoma; VEGF, Vascular endothelial growth factor; VEGFR2, Vascular endothelial growth factor receptor 2.

Progress in clinical research. The role of predictive biomarkers

Past and Recent results in drug development in GEA

Over the last decade various targeted therapies for advanced GEA have been investigated in clinical trials with largely disappointing results. As summarized in Tables 2 and 3, trastuzumab and ramucirumab (targeting HER2 and VEGFR2, respectively) are the only targeted therapies approved in gastric and GEJ cancers so far. Likewise, apatinib (TKI that selectively inhibits VEGFR2) has also been approved for Chinese population. It is noteworthy that many negative trials did not select patients by predictive biomarkers. To date, no predictive biomarkers are available for anti-VEGFR treatment and only HER2 expression has been validated as a predictive biomarker for trastuzumab in GEA.

Receptor tyrosine kinase inhibitors

Following are the receptor tyrosine kinase inhibitors

(1) *Anti-HER2:* The HER2 pathway has been well described in GC, and trastuzumab is the only validated targeted therapy in first-line setting in GC and GEJ (the ToGA trial).

Table 3
Targeted therapies for advanced GEA in second-line or more randomized phase III clinical trials.

Target	Study name/Ref.	Selected population	Drugs	mOS or mPFS	HR
HER2	GATSBY[34]	GC HER2 positive	Trastuzumab emtansine vs taxane	OS 7.9 vs 8.6 (mo)	1.15 (0.87-1.51)
EGFR	COG[35]	EC All-comers	Gefitinib vs placebo	mOS 3.73 vs 3.67(mo)	0.90 (0.74-1.09)
PARP	GOLD[36]	GC ATM negative	Taxane ± olaparib	mOS 12 vs 10 (mo)[1]	0.73 (0.40-1.34)
mTOR	GRANITE[37]	GC All-comers	Everolimus vs placebo	mOS 5.4 vs 4.3 (mo)	0.90 (0.75-1.08)
VEGFR2	**REGARD**[38]	**GC All-comers**	**Ramucirumab vs placebo**	**mOS 5.2 vs 3.8 (mo)**	**0.77 (0.60-0.99)**
VEGFR2	**RAINBOW**[39]	**GC All-comers**	**Taxane ± ramucirumab**	**mOS 9.6 vs 7.4 (mo)**	**0.80 (0.68-0.96)**
VEGFR2	[40]	**GC All-comers**	**Apatinib vs placebo**	**mOS 6.5 vs 4.7 (mo)[2]**	**0.70 (0.53-0.93)**
STAT3	BRIGHTER[41]	GC All-comers	Taxane ± napabucasin	mOS 6.93 vs 7.36 (mo)	1.01 (0.86-1.20)
DNA	**TAGS**[42]	**GC All-comers**	**TAS 102 vs placebo**	**mOS 5.7 vs 3.6 (mo)**	**0.69 (0.56-0.85)**
Antimitotic and antimicrotubule agent	[43]	**GC All-comers**	**Nab-paclitaxel 3w vs nab-paclitaxel 1w vs paclitaxel**	**mOS 10.3 vs 11.1[1] vs 10.9 (mo)[2]**	**0.97 (0.76-1.23)**

In bold, trials with positive results.
BRIGHTER, Napabucasin plus paclitaxel vs placebo plus paclitaxel in patients with pretreated advanced gastric and gastroesophageal junction adenocarcinoma; chemo, Chemotherapy.; COG, Gefitinib for oesophageal cancer progressing after chemotherapy; EC, Esophageal cancer; EGFR, Epidermal growth factor receptor; GATSBY, Trastuzumab emtansine vs taxane use for previously treated HER2-positive locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma; GC, Gastric cancer; GEJ, Gastroesophageal junction; GOLD, Olaparib in combination with paclitaxel in patients with advanced gastric cancer who have progressed following first-line therapy; GRANITE, Everolimus for Previously Treated Advanced Gastric Cancer; HER2, Human epidermal growth factor receptor 2; mOS, Median overall survival; mTOR, Target of rapamycin; PARP, Poli ADP ribosa polimerasa; RAINBOW, Ramucirumab plus paclitaxel vs placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma; REGARD, Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma; STAT3, Signal transducers and activators of transcription; TAGS, Trifluridine/tipiracil vs placebo in patients with heavily pretreated metastatic gastric cancer; VEGFR2, Vascular endothelial growth factor receptor 2.
[1] ATM-negative population.
[2] Asian population.

Following this study and unlike breast cancer, several anti-HER2 agents, for example, pertuzumab (the JACOB trial), T-DM1, MM-111 or TKI lapatinib, have failed to demonstrate survival benefit in randomized trials in HER2 positive advanced GC (see Tables 2 and 3). Divergences between gastric and breast cancer results, highlights the importance of the different molecular backgrounds in the field of targeted therapies. Moreover, intrinsic and acquired resistance to antiHER2 therapies have been reported in GEA such as, secondary driver alterations concurrent with HER2 amplification for example, PI3K pathway activation by activating PIK3CA mutations and coamplification of cell-cycle mediators, MET or EGFR[18,44] or loss of HER2 overexpression.[45] In their report, Pietrantonio et al[45] identified HER2 loss as a mechanism of acquired resistance in 32% of cases in a GEA small series of 22 matched pretreatment and postprogression samples from patients receiving chemotherapy and trastuzumab for advanced HER2-positive. All these molecular events may explain at least in part the negative results in second-line studies with anti-HER2 therapy in GC. In 2017, Doi et al, published the results of a phase I trial that addressed the safety and antitumor activity of trastuzumab deruxtecan (DS-8201), a drug conjugate targeting HER2, in patients with advanced breast and GC, showing an ORR of 50% (2/4 patients) in HER2+ patients in a salvage-line setting.[46] The role of DS-8201 in GEA patients needs to be evaluated in phase II and III trials. Margetuximab, an Fc-optimized

monoclonal antibody that targets HER2 with enhanced antibody-dependent cell-mediated cytotoxicity, is currently being studied as a potential treatment for metastatic breast cancer and GEA. No anti-HER2 agents are approved in post-trastuzumab setting in GEA. Recently, the results of the phase Ib/II study with margetuximab in combination with pembrolizumab in HER2+ GEA patients (PD-L1 unselected) have been released demonstrating acceptable toxicity and encouraging preliminary activity in second-line HER2+ GEA, with an ORR up to 30% in a highly selected population (NCT02689284).[47] This chemo-free regimen could be an interesting novel strategy for HER2+ GC which are less responsive to standard second-line treatments. Lastly, some of the anti-HER2 drugs tested in advanced disease setting are now being evaluated earlier in the disease course and for a longer period of time, for example, the INNOVATION trial from EORTC and PETRARCA trial and TRIGGER study (JCOG 1302) studied the role of trastuzumab with or without pertuzumab in perioperative setting in gastric or GEJ cancer (NCT02205047 and NCT02581462).

(2) *Anti-EGFR:* Cetuximab (the EXPAND trial) and panitumumab (the REAL3 trial) did not show benefit in advanced GEA. However, these trials were not biomarker selected and therefore any benefit from anti-EGFR therapy in sensitive patients would be diluted by the inclusion of nonsensitive patients. More recently, nimotuzumab, another anti-EGFR monoclonal antibody, also did not increase OS or PFS in the overall population in a phase II clinical trial for advanced GC, although, interestingly, a substantial benefit was observed among those patients with EGFR overexpression.[48] Likewise, an analysis of clinical outcomes regarding EGFR expression in EXPAND study patients showed a trend for improved survival and tumor response when adding cetuximab in patients with high tumor EGFR IHC scores.[49] In addition, retrospective biomarker analyses of the COG trial (gefitinib in esophagus cancer)[50] suggest that tumors with EGFR copy number gain may benefit from anti-EGFR therapy. Likewise, Maron et al reported a 58% (4/7) response rate and 100% (7/7) disease control rate to EGFR inhibitors in EGFR-amplified GEA.[51] These last studies, suggest a requirment for selection of patients by EGFR status and support that a refinement of the EGFR biomarker may achieve greater results for EGFR-targeting therapies. A phase III trial comparing nimotuzumab-IRI and IRI in EGFR overexpressed patients is currently ongoing (NCT01813253).

(3) *Anti-FGFR:* Despite interesting results in early phase trials, AZD4547, an FGFR2 TKI, did not meet pre-established efficacy criteria compared with chemotherapy in patients with GC with FGFR2 amplification/polysomy in the phase II SHINE trial.[13] Biomarker analysis from the same study demonstrated high intratumoral heterogeneity for FGFR2 gene amplification, suggesting a challenge with biomarker development. In addition, other studies have shown that simultaneous amplification of different receptor tyrosine kinases might jeopardize therapeutic efficacy of the FGFR2 inhibitor AZD4547 in FGFR2 amplified GC, which might suggest that more complex combined therapy targeting FGFR2 and other resistance-enriched receptor tyrosine kinases (RTKs) is required.[52] More recently, bemarituzumab (FPA 144-004), a new monoclonal anti-FGFR2b antibody, has been tested in GC. Bemarituzumab is glycoengineered to enhance antibody-dependent cell-mediated cytotoxicity. A phase I study of this drug identified no dose-limiting toxicities and promising antitumor activity in patients with refractory disease and high FGFR2b overexpression. Based on these results, the FIGHT trial, NCT03343301, a phase III trial with safety run-in of bemarituzumab in combination with mFOLFOX6 for FGFR2-positive advanced GC was designed. Finally, other studies are testing multi-TKIs in GC, in which inhibition includes FGFR, for example, NCT01719549 and NCT01921673. The results of these studies are not yet availbale but the inhibition of FGFR along with other kinase pathways could be another promising strategy.

(4) *Anti-MET:* Early phase trials suggested that MET-expression may serve as a predictive biomarker for anti-MET targeted therapy in GEA.[53] However, 2 monoclonal antibodies specific for HGF (onartuzumab) and MET (rilotumumab) have failed to meet expectations in phase III trials and MET-positive tumors by IHC (Table 2), suggesting that IHC alone is unreliable for selection of the target population. In contrast to the development of antibodies

specific for HGF or MET, MET TKIs have been examined in only early phase studies with promising results in GC positive for MET amplification but, to the best of our knowledge, no randomized trials with these drugs are currently underway in GEA.[54] Finally, molecular heterogeneity and receptor coamplification have been well described to cause resistance to targeted therapy in MET-amplified GC.[55]

(5) *Antiangiogenesis:* Bevacizumab, a monoclonal antibody targeting VEGF is widely used in different solid tumors, but the AVAGAST and AVATAR phase III clinical trials did not show clinical benefit of bevacizumab in advanced gastric or GEJ cancer. Bevacizumab has yielded disappointing results not only in first-line setting, but also in the curative setting, where the addition of bevacizumab to peri- operative chemotherapy did not improve survival outcomes compared to chemotherapy alone.[56] On the other hand, ramucirumab, a fully humanized monoclonal antibody targeting VEGFR2, showed significant survival benefits in second-line setting as a single agent and in combination with taxanes (REGARD and RAINBOW trials). Similarly, apatinib, a RTK inhibitor targeting VEGFR2, also demonstrated significant survival prolongation compared with placebo in a phase III with chemotherapy-refractory disease and in a Chinese GC population. However, apatinib has not been considered a cost-effective option for patients with refractory disease after cost-effective analysis.[57] It is interesting to note that ramucirumab in first-line setting failed to show any benefit in a phase II trial combined with FOLFOX and in the phase III RAINFALL trial combined with cisplatin/fluoropyrimidine. Although the study met its primary endpoint of PFS (Table 2), median overall survival and response rates did not improve with the addition of ramucirumab. Retrospective exploratory analysis from REGARD and AVAGAST studies had been published including multiple tested potential biomarkers and only Angiopoietin-2 baseline in plasma was identified as a prognostic marker for overall survival (OS) in advanced GC.[58,59] Regorafenib, an oral multitarget TKI that inhibits angiogenesis related pathways, has been tested in previously treated GC patients, suggesting a potential efficacy in a phase II trial.[60] In this study regorafenib improved mPFS from 0.9 to 2.6 mo, but only 3% of patients achieved a radiologic response. The phase III trial INTEGRATE II (NCT02773524), aims to confirm whether regorafenib is effective in prolonging survival in GC patients. To conclude, the lack of biomarker-based selection of patients for anti-angiogenic therapy and the marginal benefit demonstrated by positive trials with apatinib (1.8 mo) and ramucirumab (1.4-2-2 mo) mandate further research on biomarkers and the requirement to study novel strategies, such as drug combinations, sequencing or maintenance, to continue targeting VEGF in GEA.

Parp inhibitors

BRCA mutations are rare in GEA; however TCGA data describes frequent somatic copy-number alterations (SCNA) in esophageal and gastric carcinomas and also alterations in DNA damage repair pathways leading to high levels of homologous recombination deficiency (HRD).[61] The presence of DNA damage is associated with platinum sensitivity as well as sensitivity to poly ADP ribose polymerase (PARP) inhibitors.[62] In second-line advanced GC a randomized phase II clinical trial with olaparib (a PARP inhibitor) in combination with paclitaxel demonstrated significantly improved OS vs paclitaxel alone, both in overall population and in patients with low or undetectable ataxia-telangiectasia mutated (ATM) protein levels, an alteration associated with HRD state for whom the trial was enriched. Surprisingly, the phase III GOLD trial did not show the significant OS benefit of olaparib in the same populations (see Table 3). A different proportion in ATM-negative status between phase II and phase III and other plausible explanations might explain these disappointing results.[63] Despite the negative GOLD study there still remains an active interesting PARP inhibition in GC. Novel strategies with PARP inhibitors include maintenance after response to first line platinum therapy as surrogate for HRD, (NCT03427814), and the combination with other targeted therapies for example, anti-angiogenic drugs or immunotherapy (NCT03008278 and NCT02734004). Because of the role of PARP in DNA repair, PARP

inhibition has been shown to potentiate DNA damage induced by platinum agents, although there is concern regarding the potential for additive toxicity in combination.[64]

Stem cell inhibition

Targeting STAT3 dependent gene expression as a cancer stemness related signaling pathway appeared to be a promising strategy in GEA patients. Napabucasin (BBI-608) is a novel oral first-in-class cancer stemness inhibitor. Preclinical and early phase clinical trials showed promising antitumor efficacy signals for napabusacin in a variety of malignancies. The phase III BRIGHTER trial evaluated the efficacy and safety of combining napabucasin with paclitaxel in previously treated patients with gastric and GEJ adenocarcinoma. Unfortunately, the study failed to achieve its primary end point of OS in the intention to treat population.[41] No biomarker selection and toxicity with napabucasin, specifically diarrhea, may explain trial negative results. Analysis of secondary end points are ongoing and encompasses a biomarker analysis.[65] Other signaling pathways such as SHH (Sonic hedgehog) and Wnt/beta-catenin are dysregulated and involved in maintenance and induction of epithelial-mesenchymal transition, respectively, in gastric tumor-initiating cells. Preclinical and early phase clinical trials with different drugs targeting these pathways have been conducted with discrepant results.[66]

Novel targets

Novel treatment targets in GEA include targeting of the tight-junction protein claudins and tumor stroma modification via inhibition of matrix metalloproteinases (MMP). The phase II FAST trial[67] compared the efficacy of epirubicin, oxaliplatin, and capecitabine (EOX) with or without IMAB362 (zolbetuximab, previously called claudiximab), a first-in-class anti-claudin18.2 antibody, as first-line therapy in patients with high expression of claudin18.2 by IHC, in gastric and GEJ adenocarcinoma. The FAST study demonstrated gains in PFS and OS for the EOX plus IMAB362 arm and justifies moving to phase III trial (NCT03504397 and NCT03653507). Andecaliximab, GS-5745, is a monoclonal antibody inhibitor of matrix metalloproteinase 9 (MMP9), an extracellular enzyme involved in matrix remodeling, angiogenesis, tumor growth, and metastasis. Preclinical studies demonstrate that MMP-9 inhibition alters the tumor microenvironment, which is associated with greater chemotherapy penetration and improved antitumor immunity. Results from the phase III GAMMA-1 study evaluating modified FOLFOX with or without GS-5745 in gastric and GEJ adenocarcinoma have been recently reported in ASCO 2019 GI Cancers Symposium (NCT02545504). Unfortunately, the study did not meet its survival end points but an exploratory analysis suggested that survival outcomes were significantly improved in patients aged 65 years or older. This finding deserves further study. Mouse studies do show that serum MMP9 can increase with age, though data in humans are limited.

Novel cytotoxic drugs

Novel cytotoxic drugs in GEA include TAS 102, nab-paclitaxel and TAS 118. In the phase III TAGS trial, TAS 102, an oral combination of trifluridine/tipiracil, a nucleoside analogue, significantly improved OS vs placebo in patients with heavily pretreated gastric and GEJ adenocarcinoma. TAS 102 has been established as a new therapy option in chemotherapy-refractory gastric and GEJ cancer.[42] However, TAS 102 is largely disease stabilizing with no responses seen. Weekly nab-paclitaxel showed noninferiority to soluble-based paclitaxel as second-line chemotherapy for advanced GC in terms of OS in a phase III Japanese trial.[43] TAS 118 (S1 plus leucovorin) is being evaluated in a phase III trial in Asian countries (NCT02322593).

Immunotherapy

In September 2017, FDA approved pembolizumab for the treatment of patients with recurrent, locally advanced or metastatic, gastric or GEJ adenocarcinoma, whose tumors expressed PD-L1 and with disease progression on or after 2 or more systemic therapies. The approval was based on the results of the KEYNOTE-059/cohort 1 trial (see Table 4). Simultaneously, nivolumab was registered as third-line treatment in Japan based on the results of ATTRACTION-02 trial (see Table 4). However, these initial good results have been followed by the results of 2 large negative trials that have shown that immune checkpoint blockade is not superior to chemotherapy in the second-line setting or beyond in unselected or low PD-L1 expressing patients.[68,69] Table 4 summarizes the results of selected trials with immune checkpoint inhibitors in GEA. Of note, some of pembrolizumab trials and all nivolumab GEA trials enrolled patients with PD-L1 positive and negative tumors with responses seen in both cohorts. It is also important to highlight that different antibodies and scoring systems have been used in the trials and that none of them have stratified or selected patients by any other molecular criteria than PD-L1 expression that could usefully detect responders from nonresponders. For example, in esophageal cancer, KEYNOTE-181 study (see Table 4) evaluated pembrolizumab vs chemotherapy as second-line therapy including both histologies: Squamous cell carcinoma (two-thirds of population) and adenocarcinoma (one-third of population) using a higher combined positive score. In this study pembrolizumab did not improve OS in the whole population, vs chemotherapy, but did improve survival for patients with strong expression of PD-L1 (combined positive score $\geq$ 10). Overall, response rates of immune checkpoint inhibitor monotherapy in GEA are around 10%-25% depending on the number of previous lines of chemotherapy and PD-L1 status, with higher responses reported in distinct subgroups such as MSI-H, EBV or high PD-L1 expression.[68,70] Therefore, combinatorial strategies may improve these outcomes. The phase I/II CheckMate-032 trial evaluates the combination of anti-PD-1 and anti-CTLA-4 and demonstrates improvements in objective response rates when compared to single-agent anti-PD-1 therapy, however, also with increased rates of toxicity (Table 4). Another combination of significant interest is the combination of anti-angiogenic and anti-PD-1/L1 directed drugs. Preclinical data suggests that blocking VEGFR-2 and the PD-1/PD-L1 pathway induces synergic antitumor effects. Thus, ramucirumab plus pembrolizumab, durvalumab or nivolumab have been studied in phase I/II trials with no unexpected toxicities and demonstrated antitumor activity.[71-75] Two phase III trials evaluating checkpoint inhibitors combined with chemotherapy in first-line setting are ongoing (KEYNOTE-590-NCT03189719 and CheckMate-648-NCT03143153 in esophageal cancer). The results of KEYNOTE-062-NCT02494583 in GC have recently been reported in abstract form suggesting a benefit to pembrolizumab monotherapy in patients with high PD-L1 expressing tumours only. JAVELIN Gastric 100 results, avelumab[HYPHEN]maintenance treatment, did not meet its primary objective of demonstrating superior OS in the randomized or PD[HYPHEN]L1+ population.[76] Finally, in locally advanced disease, additional trials with checkpoint inhibitors in combination with chemoradiation are being studied in GEA patients (NCT02735239 and NCT02730546).

Challenges in molecular biology and clinical research-tying it all together

Applying TCGA molecular subtypes to treatment

None of the so-called traditional GC classifications based on histopathology has proved to be useful for treatment selection thus far. Up to now, design of clinical trials with GEA did not account for analysis of molecular subtypes or drivers, which might be desirable in future. There are other tumor scenarios where the molecular classification is already used to select specific treatments for patients by matching patients to a particular clinical trial based on their specific molecular profile. For example, the MoTriColor project consists of 3 phase II studies based on solid rationales for specific subgroups of patients with advanced colorectal

Table 4
Summary of the results of selected trials with immune checkpoints inhibitors in Gastric and Esophageal cancers.

Checkpoint Inhibitor	Target	Phase	N/Esophageal or Gastric disease/Tumor and Patient Characteristics/ Strategy	Trial name and Reference	Results
Pembrolizumab	PD-1	Ib	36 pts/Gastric/PD-L1+/pretreated pts	KEYNOTE-012[77]	ORR 22%, median duration of response 40 w, mOS 11.4 m mPFS 1.9 m
		Ib	23 pts/Esophageal cohort (ESCC, EAC and GEJ)/PD-L1+/monotherapy/pretreated pts	KEYNOTE-028[78]	ORR 30%, median duration of response 15 mo
		II Multi-cohort	Cohort 1 (259 pts/Gastric/any PD-L1 status/monotherapy/ pretreated pts	KEYNOTE-059 Cohort 1[79]	ORR 11.2% (overall) **ORR 23% (PD-L1+)** ORR 8.6% (PD-L1-). Duration of response (8, 16 and 7 mo). ORR in MSI subgroup 57%.
			Cohort 2 (25 pts/Gastric/any PD-L1 status/combination with first-line chemo.)	Cohort 2[80]	ORR 60% (all pts). ORR 68% (PD-L1+) ORR 37% (PD-L1-)
			Cohort 3 (31 pts/Gastric/PD-L1+/monotherapy in first-line setting)	Cohort 3[81]	Preliminary results: ORR 26%. mOS 20.7 mo
		III	592 pts/Gastric/PD-L1+/pembrolizumab vs paclitaxel in second-line setting	KEYNOTE-061[68]	mOS 9.1 vs 8.3, HR 0.82 (0.66-1.03) $P = 0.042$
		II	121 pts/Esophageal (ESCC, EAC and GEJ)/any PD-L1 status/monotherapy/pretreated pts	KEYNOTE-180[75]	ORR 10% (all patients) ORR 14% (ESCC), ORR 5% (EAC), ORR 14% (PD-L1+), ORR 6% (PD-L1-)
		III	628 pts/Esophageal (ESCC, EAC and GEJ)/any PD-L1 status/pembrolizumab vs standard therapy after first-line therapy	KEYNOTE-181[82]	mOS (ITT) 7.1 vs 7.1 HR 0.89 and mOS (ESCC) 8.2 vs 7.1 HR 0.78 mOS (PD-L1+) 9.3 m vs 6.7 m; HR 0.69 (0.52-0.93) $P = 0.0074$
Nivolumab	PD-1	I/II	160 pts/Gastric/Non-Asian population/any PD-L1 status/pretreated pts/Nivo monotherapy and 2 combinations Nivo (N3) + Ipilimumab at different dose levels (N1:I3 and N3:I1)	CheckMate-032[83]	ORR 12%, 24%, 8% (all pts) and ORR 19%, 40%, 23% (PD-L1+) mOS 24 mo (all pts): 22, 22, NR mOS 18 mo (PD-L1+): 13, 50, 15
		III	493 pts/Gastric/Asian population/any PD-L1 status/pretreated pts/Nivo vs placebo.	ONO-4538-12 ATTRACTION-02[84]	ORR 11% vs 0% **12-mo OS 26.6% vs 10.9%**
Ipilimumab	CTLA-4	I/II	160 pts/Gastric/Non-Asian population/any PD-L1 status/pretreated pts/Nivo monotherapy and 2 combinations Nivo + Ipilimumab	CheckMate-032[83]	ORR 12%, 24%, 8% (PD-L1-) and ORR 19%, 40%, 23% (PD-L1+)
		III	499pts/Gastric/any PD-L1 status/maintenance avelumab vs chemo	JAVELIN	mOS 10.4 vs 10.9, HR 0.91 (0.74-1-11)

(*continued on next page*)

Table 4 (*continued*)

Checkpoint Inhibitor	Target	Phase	N/Esophageal or Gastric disease/Tumor and Patient Characteristics/ Strategy	Trial name and Reference	Results
Avelumab (MSB0010718C)[76]	PD-L1	Ib	75 pts/Gastric/Asian population/ any PD-L1 status/2 cohorts: Pretreated (Pre) pts and maintenance (Mn)	JAVELIN[85]	ORR 15% (Pre) and 7 % (Mn) mPFS 11.6 w and 11.6 w (PD-L1-) mPFS 36 w and 17.6 w (PD-L1+)
		III	371 pts/Gastric/avelumab vs irinotecan or taxanes in third-line setting	JAVELIN 300[69]	mOS 4.6 vs 5, HR 1.1 (0.9-1.4)
Durvalumab (MEDI4736)	PD-L1	I	Gastric cohort 16 pts/any PD-L1 status	[86]	ORR 25%
Atezolizumab (MPDL3280A)	PD-L1	I	Gastric cohort 1 pt	[87]	1 pt with PR
Tremelimumab	CTLA-4	II	18 pts/Gastric/second-line	[88]	1 pt with PR and 4 pts with SD

In bold results that lead to registration.
ATTRACTION-02, Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least 2 previous chemotherapy regimens.; chemo, Chemotherapy; EAC, Esophageal adenocarcinoma; ESCC, Esophageal squamous cell carcinoma; GEJ, Gastroesophageal junction; Ipi, Ipilimumab; JAVELIN, Studies of avelumab; KEYNOTE, Studies of pembrolizumab-Keytruda; mOS, Median overall survival; 12-mo OS, 12-month overall survival; mPFS, Median progression-free survival; Nivo, Nivolumab; ORR, Overall response rate; PR, Partial response; pt, Patient; pts, Patients; SD, Stable disease.

cancer: (1) Combination of chemotherapy and a TGF-beta inhibitor in patients presenting a C-type(mesenchymal) signature; (2) Vinorelbine in patients with a BRAFm-like signature; and (3) An anti-PD-1 drug in combination with bevacizumab in patients with a MSI-like signature.[89] Meanwhile, only a few clinical trials are currently evaluating specific treatment strategies for most TCGA immunogenic subtypes: MSI and EBV (NCT03257163 and NCT02488759). However, because of MSI and EBV innate positive prognostic value, these subtypes are found much less frequently in patients with advanced than with non-advanced disease for example, MSI-H tumors were/represented 4% of the KEYNOTE-059 cohort 3.[81]

CIN subtype

The CIN subgroup represents the largest group in the TCGA analysis, accounting approximately 50% of the GC cases examined in the study. This subgroup comprises 65% of GEJ tumors and correlates well with the intestinal-type phenotype. CIN tumors show marked aneuploidy and high frequency of genomic amplifications of oncogenes such as the RTK ERBB2, EGFR, MET or FGFR2 (known actionable targets) and VEGFA, cell cycle mediators such as CCND1, CCNE1, and CDK6; and transcription factor oncogenes such as GATA4, GATA6, and MYC. Hence, in this group it is worthwhile to explore targeted therapy according to specific/existing RTK amplification, as well as antiangiogenic therapy and cyclin-dependent kinase (CDK) inhibitors. Moreover, and as commented before, cancer types with a large number of SCNA have a larger number of SCNAs in DNA damage repair pathways, a molecular feature associated with sensitivity to PARP inhibitors.

EBV subtype

Tumors positive for Epstein-Barr virus (EBV) represent approximately 10% of the GC cases examined in the TCGA study. The main characteristic of this subgroup was high levels of DNA promoter hypermethylation; all EBV positive tumors demonstrated *CDKN2A* (p16) promoter

methylation. The association between CDKN2A/B deletion and the sensitivity to palbociclib, an FDA-approved CDK4/6 inhibitor, has been demonstrated in many cancers.[90] In addition to the DNA hypermethylation status, EBV subgroup displayed a high prevalence of other clinically interesting features, some of them actionable such as highly recurrent *PIK3CA* mutations (in approximately 80% of EBV positive tumors) and elevated expression of Janus kinase 2 (*JAK2*) and programmed death ligands 1 and 2 (PD-L1 and PD-L2), as a result of an amplification of the short arm of chromosome 9 (amplification at 9p24.1) (Table 1). These molecular features suggest a role for not only CDK inhibitors, but also PI3KCA inhibitors, JAK2 inhibitors, PD-L1/PD-L2 inhibitors, and, possibly, DNA hypomethylating agents. Interestingly, mutations in *ARID1A* were also present in more than 50% of EBV positive tumors, by contrast, few EBV positive tumors had *TP53* mutations (the most frequently mutated gene in GC). Loss-of-function mutations in *ARID1A* disrupt DNA mismatch repair (MMR) and improve outcomes for mice treated with anti-PD-1.[91] TCGA reports that 80% of EBV tumors and 42% of MSI tumors harbor PIK3CA mutations. Clinical trials targeting PI3K pathway in unselected GC patients have failed to demonstrate benefit in survival (Table 3). However, (1) patients with *PIK3CA* or *PTEN* mutations presented a higher response rate when compared with patients without the mutations; (2) preclinical work has demonstrated that *PIK3CA* mutations lead to a constitutive activation of the PIK3CA signaling pathway in the absence of growth factors; and (3) persistent PIK3CA signaling is a significant component of acquired resistance to upstream inhibitors.[92] Taking into account the aforementioned points, specific treatment strategies for *PIK3CA* mutated GCs are worth exploring (NCT02451956).

MSI subtype

The MSI subgroup represents approximately 20% of the GC cases examined in the TCGA study. MSI subtype is characterized by hypermethylation resulting in MutL homolog 1 (MLH1) silencing and high mutation rates. Hypermutation status generates a large number of neoantigens. The high degree of mutational burden as well as tumor-specific neoantigens and the mismatch-repair deficiency status predict a good response to an anti-PD-1/PD-L1 treatment strategy. Mutations in *ARID1A*, another sensitising event for anti-PD-1/PD-L1, were also present in 44% of MSI tumors. MSI subgroup generally lacked targetable amplifications, but targetable mutations in *ERBB1-3* and *PIK3CA* (up to 42%) were observed, and therefore, molecules targeting these pathways are also promising therapeutic candidates for this subgroup. HER2 mutations, are less frequent than HER2 amplification but some reports describe them as activating mutations associated to response to existing HER2-targeted drugs.[93] Regarding HER3 mutations, the upregulated activity of HER3 has been associated with tumor resistance to therapeutic agents targeting EGFR or HER2.[94] Interestingly, a “basket” trial including a GEA cohort in patients with HER2 or HER3 mutated cancers has been published using the pan-HER tyrosine kinase inhibitor neratinib. In this trial neratinib activity was influenced by both tumor lineage and mutation type with single-agent neratinib activity in some cohorts such as breast cancer cohort and no clinical activity in colorectal and GEA cohorts (only 5 patients) and in HER3 mutations.[95] The small number of patients in the GEA cohort limits the interpretation of these results. Finally, as we have mentioned before, some reports have suggested MSI as a marker for lack of benefit from perioperative chemotherapy.

GS subtype

The GS subgroup represents approximately 20% of the GC cases examined in the TCGA study, and it was enriched with diffuse-type adenocarcinomas. Although the definition of this subgroup is based on the lack of other subtype features, several unique molecular alterations have been described by TCGA in this subgroup; these are mutations in *RHOA* (Ras homolog gene family, member A), mutations in *CDH1* (gene encoding E-cadherin) and fusions involving RHO-family GTPase-activating proteins. These alterations promote a lack of cell adhesion, morphologic

changes and increased migratory activity of GC cells that may account for the discohesive, invasive nature of diffuse GC and GS subtype. So far, there is no available targeted drug against RHOA mutations or fusions. However, RHOA and its oncogenic signaling pathway, represent a valuable signpost for development of effective treatments for diffuse GC. There are currently no direct inhibitors of RHOA in clinical use. In addition, recurrent CLDN18-ARHGAP fusions were reported by TCGA in 15% of GS subtype. Interestingly, these fusions and RHOA mutations were found to be mutually exclusive. The CLDN18-ARHGAP fusions represent an ideal drug target candidate because of its accessibility on the cell membrane and its complete absence in nonmalignant cells. In fact, as commented before, an anti-CLDN18 monoclonal antibody (IMAB362), has been developed for GC. With respect to other pathways, as also observed in the CIN subtype, the presence of *FGFR2* and *VEGFA* amplifications in GS tumors suggests FGFR2 and angiogenesis as an attractive targetable pathway. TCGA also reported frequent mutations in *ARID1A* gene and as we have commented above, these findings may suggest a potential role of anti-PD-1/PD-L1 inhibitors in this group.[91]

EAC

As commented before, TCGA showed that despite subtle differences in the frequencies at which some genetic alterations arise, there is a notable molecular similarity between EAC/GEJ and CIN GCs. Moreover, defects in homologous recombination and sensitising events for CDK4/6 inhibitors, such as *CDK6* amplifications and *CDKN2A* deletions, described in EAC in up to 20% and more than 50% of the samples respectively[4,5] suggest that PARP inhibitors and CDK4/6 inhibitors might be beneficial in these subgroup of EAC patients.

Challenges of heterogeneity in gastroesophageal cancer

The heterogeneity of GEA represents a major obstacle for biomarkers discovery and targeted treatment development. Up to now and regardless the significant anatomical, histologic, epidemiologic, geographic, and molecular diversity we generally approach GC and EC as a single disease. One of the major hindrances to overcome this problem has been the lack of effective methods for evaluating intratumor heterogeneity. Liquid biopsies by ctDNA profiling could potentially offer an alternative for tissue biopsies analysis that carries the risk of a nonrepresentative result of the whole disease. Pectasides et al found a 87.5% concordance for targetable alterations in metastatic tissue and ctDNA in discordant primary and metastatic lesions[16] suggesting that ctDNA sequencing could detect genomic alterations present in metastases but not in the primary tumour. Similarly, Pearson et al showed that high-level clonal *FGFR2* amplification, a low prevalence alteration in GEA and predicting response to FGFR-selective inhibitors, can be detected through ctDNA screening.[14] Finally, Sanchez-Vega et al[18] in their study assessing resistance to afatinib in GEA patients commented before, demonstrated the viability of detection of loss of *EGFR* amplification and gain of *MET* amplification by ctDNA as a potential mechanism of acquired resistance to the drug. However, liquid biopsy alone is not able to fully overcome the impact of tumor heterogeneity; this will require a combination of other features that encompass the development of novel clinical trial designs and therapeutic strategies. New clinical trial designs such as the PANGEA trial (Personalized Antibodies for GEA, NCT02213289)[17] address not only tumor molecular heterogeneity, but also the accrual difficulties of GEA trials exacerbated by low frequencies of molecular "oncogenic drivers." PANGEA is a phase II trial for metastatic or recurrent GEA in which patients are treated with chemotherapy plus a biologic agent based on biomarker profiling (HER2positive: Trastuzumab; METpositive: None, FGFR2positive: None; EGFR: ABT806; MSI-H: Nivolumab; "RAS like": Ramucirumab). This biomarker profiling was performed on primary tumor and metastatic lesions as well as at baseline and first and second progression and determines a change in the biologic agent if the molecular category evolves.

Novel therapeutic strategies to tackle heterogeneity matter include targeting cancer stem cell-like (as previously commented) and genomic instability, both being sources of the heterogeneity. Finally, other therapeutic strategies such as adaptive therapy with treatment holidays, intermittent dosing schedules (on-off cycles) or reduced drug doses, rather than using the maximum tolerated dose, have been described as a potential solution to avoid rapid emergence of drug-resistant subclones.[96,97] This hypothesis is supported by Pectasides and Sanchez-Vega reports that give the rapid selection for or against driver amplifications presented at baseline and heterogeneously within an individual patient as one of the explanations for limited efficacy of targeted kinase inhibitors in GEA.

East West divergence

The incidence and mortality rates of GEA vary according to geographical regions.[1] In GC the highest incidence is in Eastern Asian countries, however the same countries have consistently reported better treatment outcomes. These divergences have been a challenge for drug development. The AVAGAST study[33] which analyzed the addition of bevacizumab to first-line therapy in advanced GC, did not meet its primary end point of OS. However, a preplanned subgroup analyses showed a regional variability in these results; bevacizumab prolonged survival for patients enrolled in North and Latin America, but not for those patients in Asia. Gene expression profiling was not performed in AVAGAST study and the reasons behind this paradox remain unclear. However, regional significant differences in healthcare environment like greater use of second-line chemotherapy and screening programs in Asia may have contributed to the differences in prognosis observed in the AVAGAST study[98] introducing a bias and contributing to differences in survival benefit from bevacizumab in the study. This is reflected in RAINBOW trial, in which addition of ramucirumab resulted in improvements in PFS and RR for patients from Asia, but no signicant improvement in OS. More recently, KEYNOTE- 181 also demonstrated increased efficacy of pembrolizumab in esophageal cancer patients in Asia. Even in high PD-L1 expresssing tumors, the benefit of pembrolizumab as compared to chemotherapy seemed to be higher in Asian patients, according to a subgroup analysis. TCGA reported some differences in pathway-level gene expression between patients from Eastern Asia compared with patients from other regions.[2] Another study revealed differences in tumor immunity between tumors from Asian and non-Asian patients; non-Asian GCs were associated with enrichment of tumour infiltrating T-cells as well as T-cell gene expression signatures, including CTLA-4 signalling.[99] Moreover, Asian Cancer Research Group showed that the proportion of patients varied according to the geographic region when their classification to other cohorts such as TCGA cohort was applied.[100]

Conclusion

The extensive molecular characterization of GEA provided by TCGA network and other research groups has changed our understanding of this disease/entity. Nevertheless, as of today, design of clinical trials with GEA do not account for analysis of molecular subtypes or “oncogenic drivers.” In future, patients are likely to be selected for targeted therapy by the presence or absence of specific molecular characteristics rather than by morphological phenotype or even site of origin. Classical trial designs in GEA are challenged by heterogeneity, a historically low frequency of oncogenic drivers (although new drivers are emergent),[5] and scarcity of tissue. Intratumoral heterogeneity might have significant impact on clinical outcomes in GEA and influence the selection of suitable patients for targeted therapy and consequently their results. Moreover, selecting patients by biomarker status and refining biomarkers taking into account the well described reasons for intrinsic and acquired resistance may lead to better results in targeted therapies. Strategies such as the combination of inhibitors guided by secondary driver events should be evaluated in clinical trials. Performing liquid biopsy could overcome some weaknesses of a

single baseline tissue biopsy and potentially reduce the need for costly and invasive metastatic biopsies. However, incomplete overlap between primary tumour, metastases and liquid biopsy has been described and these methods may be currently considered complementary. With respect to immunotherapy, and given the good results in MSI-H or EBV positive tumors, anti-PD-1 therapy should be actively considered in this patient population. Finally, molecular heterogeneity and tumor immunity differences in patients from different regions should be considered in design of future GEA trials.

Authors' contribution

Serra O, Smyth EC and Lordick F conceived the idea, performed the research and wrote the paper.

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