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MINIREVIEWS

Targeted therapies in metastatic gastric cancer: Current knowledge and future perspectives

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Abstract

Gastric cancer (GC) represents a leading cause of cancer related morbidity and mortality worldwide accounting for more than 1 million of newly diagnosed cases and thousands of deaths every year. In the last decade, the development of targeted therapies and the optimization of already available chemotherapeutic drugs has expanded the available treatment options for advanced GC and granted better survival expectations to the patients. At the same time, global efforts have been undertaken to investigate in detail the genomic and epigenomic heterogeneity of this disease, resulting in the identification of new specific and sensitive predictive and prognostic biomarkers and in innovative molecular classifications based on gene expression profiling. Nonetheless, several randomized studies aimed at exploring new innovative agents, such as immune checkpoint inhibitors, failed to demonstrate clinically meaningful survival advantages. Therefore, it is essential to further improve the molecular characterization of GC subgroups in order to provide researchers and medical oncologists with new tools for patients' selection and stratification in future clinical development programs and subsequent trials. The aim of the present manuscript is to provide a global overview of the recent molecular classifications from The Cancer Genome Atlas and the Asian Cancer Research Group and to present key promising developments in the field of immunotherapy and targeted therapies in metastatic GC.

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Core tip: Gastric cancer (GC) still represents a leading cause of cancer related morbidity and mortality worldwide accounting for more than 1 million of newly diagnosed cases and thousands of deaths every year. In the last decade, global efforts have been undertaken to investigate in detail the genomic and epigenomic heterogeneity of this disease, resulting in innovative molecular classifications of GC based on gene expression profiling and in the identification of new specific and sensitive predictive and prognostic biomarkers. At the same time, the development of targeted therapies has expanded the treatment scenario for advanced GC. The aim of the present manuscript is to provide a detailed and comprehensive overview of the recent molecular classifications from The Cancer Genome Atlas and the Asian Cancer Research Group and to present key promising developments in the field of immunotherapy and targeted therapies in metastatic GC.

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INTRODUCTION

Gastric Cancer (GC) is the third leading cause of cancer death and the fifth most common malignancy worldwide for both sex, accounting for over 1 million new diagnoses and almost 800000 patients deaths in 2018[1]. Over 70% of GCs occur in low/middle income countries with the highest rates in Eastern Asia, Eastern Europe and South America and the lowest rates in North America and Western European countries[2,3].

Since the middle of the 20th century a progressive decline of distal GC incidence has been observed, possibly due to recognition and eradication of certain risk factors such as *Helicobacter pylori* (*H. pylori*), the introduction of refrigerators and an increased use of fresh food[4,5]. Conversely, the rate of gastroesophageal junction cancer (GEJC) is increasing in Western countries, which probably reflects the increase of gastroesophageal reflux disease and visceral obesity[6,7]. Considering the rising of worldwide population, the absolute number of new diagnoses per year is increasing, so that GC still remains an important cause of cancer-related mortality and a main global health-care problem.

Surgery is the only potentially curative treatment while neoadjuvant and adjuvant therapies should be integrated with surgery in locally advanced disease. Despite these multimodal treatments the 5 years overall survival is less than 30%, and in metastatic setting the prognosis remains poor with a median overall survival (OS) of 1 year[8].

The last decade has been characterized by a better understanding of molecular mechanisms of pathogenesis and biology of GC with the definition of new genomic classifications and identification of prognostic and predictive biomarkers potentially predictive of response to innovative target agents. Despite this, up to now, few target-directed options have been approved for metastatic GC. The anti-human epidermal growth factor receptor-2 (HER2) drug trastuzumab has been the first target agent approved for HER2 high expressing advanced GCs and GEJCs, while the antiangiogenic drug ramucirumab has received approval in the second-line setting as a monotherapy or in combination with paclitaxel. More recently, anti-PD1 agents such as nivolumab and pembrolizumab have been approved for patient with heavily pretreated advanced GC in some Asian countries and North America, respectively.

More than 90 percent of GCs are adenocarcinomas. According to Lauren's criteria, gastric adenocarcinomas are divided into intestinal (54%), diffuse (32%), and indeterminate type (15%), which present distinct epidemiology, histological appearance, cell differentiation and molecular pathogenesis[9,10]. Intestinal carcinomas are more likely to be sporadic than inherited and causally related to *H. pylori* infection

and the Correa's phenotypic multistep cascade (*i.e.*, longstanding gastritis, atrophic gastritis, dysplasia and adenocarcinoma)[11]. Histologically, diffuse-type adenocarcinomas are poorly differentiated and composed by discohesive cells usually characterized by with a dysregulation in the expression of E-cadherin, a key cell surface and connection protein[12]. Both Lauren classification and the World Health Organization (WHO) 2010 classification[13], although widely used in the clinical practice, remain insufficient to guide precision treatments for the individual patient and GC histotype is not a parameter considered in the treatment decision process.

GC MOLECULAR CLASSIFICATION

The Cancer Genome Atlas research group

In 2014, the The Cancer Genome Atlas (TCGA) network proposed a comprehensive molecular analyses of 295 primary GC based on array-based somatic copy number analyses, whole exome and genome sequencing, messenger RNA and microRNA sequencing, and reverse-phase protein array profiling. As a result, four GC subgroups were identified: Epstein-Barr (EBV) positive tumors, tumors with microsatellite instability (MSI), genomically stable tumours (GS) and tumors with chromosomal instability (CIN)[14].

EBV activation was found in about 9% of tumor samples. All EBV positive tumours were associated to extreme DNA hypermethylation with high levels of CIMP (*i.e.*, CpG island methylation) of *CDKN2A (p16 NK4A)* promoter but not of *MutL homolog 1* (*MLH1*), characteristic of MSI associated CIMP. As previously reported, phoshatidylinositol-4-5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*) mutations were detected in 80% of EBV-positive GC[15,16]. Moreover, the finding of an overexpression of programmed death ligands 1 and 2 (PD-L1 and PD-L2) and of a significant immune cell presence supported the rationale to evaluate checkpoint inhibitors in this GC subgroup.

The MSI group (22%) was characterized by loss of mismatch repair functions which lead to alterations in length of repetitive regions in DNA known as microsatellites. The hypermethylation of *MLH1* promoter region was the most representative mismatch repair defect in patient with MSI sporadic GCs. Alterations of *PIK3CA*, *ERBB3* and *ERB22* were found and in contrast to MSI colorectal cancers, *BRAF* mutations have never been described in MSI-GCs. MSI GCs can be part of the spectrum of inherited malignancies such as Lynch syndrome and nonpolyposis colorectal cancer syndrome which are associated to inherited germline mismatch repair defects[17]. Although colorectal and endometrial cancers are the most common cancer associated to these syndromes, other extracolic tumours including GC, can occur[18]. MSI GCs are mainly associated with intestinal histotype, are localized in the antrum, with less frequent lymph-node involvement, occur mainly in elderly age and have a more favourable prognosis[19,20].

GS tumors (20%) are characterized by low copy number alterations and a low mutation rate. *ARID1*, *RHOA* and *CDH1* mutations are the principal somatic genomic alterations observed in this class. An interchromosomal translocation between *CLDN18* and *ARHGAP26*, mutually exclusive from RHOA mutations, was found in the 15% of GS GCs. These tumors usually occur in younger patients (median age 59), and are correlated with diffuse histology and distal localization. GS subtype was associated with the worst OS and prognosis among the four TCGA subtypes.

The fourth TCGA group are GCs with CIN (50%) characterized by DNA aneuploidy, highly variable chromosomal copy numbers, and mutations of the tumor suppressor *TP53*, which is responsible for chromosomal instability. Frequent genomic amplifications of receptor tyrosine kinases (RTKs)/RAS pathway were found, including *epidermal growth factor receptor* (*EGFR*), *ERBB2*, *ERBB3*, MET proto-oncogene (*MET*), *fibroblast growth factor receptor 2* (*FGFR2*), *vascular endothelial growth factor A* (*VEGFA*), and *KRAS*. Most of CIN GCs are classified as intestinal-type, frequently located at the gastroephageal junction/cardia[21,22].

The Asian Cancer Research Group

In 2015 the Asian Cancer Research Group (ACRG) proposed a different molecular classification based on gene expression profiling, genome-wide copy number microarrays and targeted gene sequencing of 300 primary tumors with the definition of four molecular subtypes: Microsatellite unstable type, epithelial to mesenchymal-like type (MSS/EMT), MSS/TP53 and MSS/TP53 negative subtypes[23]. Each of these molecular subtypes was correlated to distinct clinical phenotypes and genomic alterations.

MSI GCs occurred preferentially in the antrum, with intestinal histology (more than

60%) and half of them were diagnosed at an early stage disease. MSI tumors were characterized by loss of expression of *MLH1* and an elevated DNA methylation signature. The MSI subtype was associated with the presence of hypermutation, with mutations of *ARID1A* (44.2%), the *PI3K-PTEN-mTOR* pathway (42%), *KRAS* (23.3%) and *ALK* (16.3%).

The MSS/EMT subtype was observed at significantly younger age, with diffuse histology at stage III/IV and showed *CDH1* loss of expression. The EMT subtype presented a lower number of mutation events when compared to the other MSS groups. The MSS/EMT had the worst prognosis, while the MSI subtype showed the best prognosis of the four. The authors observed that the MSS/EMT group presented a higher percentage of recurrence *vs* the MSI group (63% *vs* 23%). The MSS/EMT GC subtype was associated to a higher frequency of peritoneal metastases compared to all other subtypes, while a higher percentage of liver-limited metastasis in the MSI and MSS/TP53 subtypes was found.

MSS/TP53 positive and MSS/TP53 negative showed an intermediate prognosis and also an intermediate chance of recurrence. EBV infection was more frequently associated to MSS/TP53 positive group. MSS/TP53 negative subtype exhibited the highest prevalence of *TP53* mutations (60%) and a low frequency of other mutations, as well as recurrent focal amplification of *ERBB2*, *EGFR*, *CCNE1*, *CCND1, MDM2, ROBO2, GATA6* whereas the MSS/TP53 positive subtype showed a relative higher (compared to MSS/TP53 negative) of mutations in *APC*, *ARID1A*, *KRAS*, *PIK3CA* and *SMAD4*.

Comparison between the TCGA and ACRG classification

Comparisons between TCGA and ACRG genomic subtypes showed similarities and differences (Figure 1). Both molecular classifications revealed MSI positive tumors and TCGA GS, EBV and CIN subtypes are likely to be approximated to ACRG MSS/EMT, MSS/TP53 positive and MSS/TP53 negative subtypes, respectively. Tumors classified as the GS subtype in the TCGA set were present across all ACRG subtypes in the ACRG data set, while tumors classified as the TCGA CIN subtype were present across all ACRG subtypes in the TCGA data set. A substantially lower percentage of Lauren's diffuse subtype cases were found in the TCGA cohort compared to ACRG database (24% *vs* 45% respectively) with the majority (57%) of Lauren's diffuse-sub-type cases present in the TCGA GS group but only 27% cases present in the ACRG MSS/EMT subtype. Additionally, *CDH1* and *RHOA* mutations, which were mutated in TCGA GS, were infrequent in the ACRG MSS/EMT subtypes. These differences suggest that TCGA GS type is not equivalent to the ACRG MSS/EMT subtype.

Collectively, these findings confirm that the TCGA and ACRG classification systems are related but distinct in terms of demographics, molecular mechanisms, driver genes and prognosis. Although these novel classifications have provided a deeper understanding of GC biology, some limitations can be observed. First, these analyses are based on complex molecular technologies and could not be replied in standard laboratories. Furthermore, a prospective validation on large scale including patients of different age and ethnicity is needed. Finally TCGA and ACRG classifications are the result of comprehensive molecular analysis on malignant GC epithelial cells that don't consider the impact of peritumoral stromal cells. Of note, novel stromal gene signatures have been analyzed in comparison to the dominant cancer phenotypes identified by TCGA and ACRG, revealing distinct stromal phenotypes[24,25].

CURRENT STANDARD TREATMENTS IN METASTATIC GC

Chemotherapy remains the backbone of therapy in patients with metastatic GC and good performance status. Available data from randomized clinical trials showed a statistically significant benefit of palliative chemotherapy, compared with best supportive care (BSC), in terms of symptom control, improvement of quality of life (QoL) and overall survival (OS).

In the first line setting a variety of cytotoxic drugs, including platinum compounds, fluoropyrimidines, anthracyclines, taxanes, and irinotecan, have shown activity in GC. Currently, a combination including a platinum compound (cisplatin or oxaliplatin) plus a fluoropyrimidine (5-FU,capecitabine, or S-1) agent is one of the most widely used doublet on the basis of a balanced benefit-to risk ratio[26].

Currently, trastuzumab is the only molecularly targeted drug accepted in first-line therapy, in combination with cisplatin and a fluoropyrimidine, for the treatment of patients with metastatic HER2-overexpressing GC or GEJC who have not received

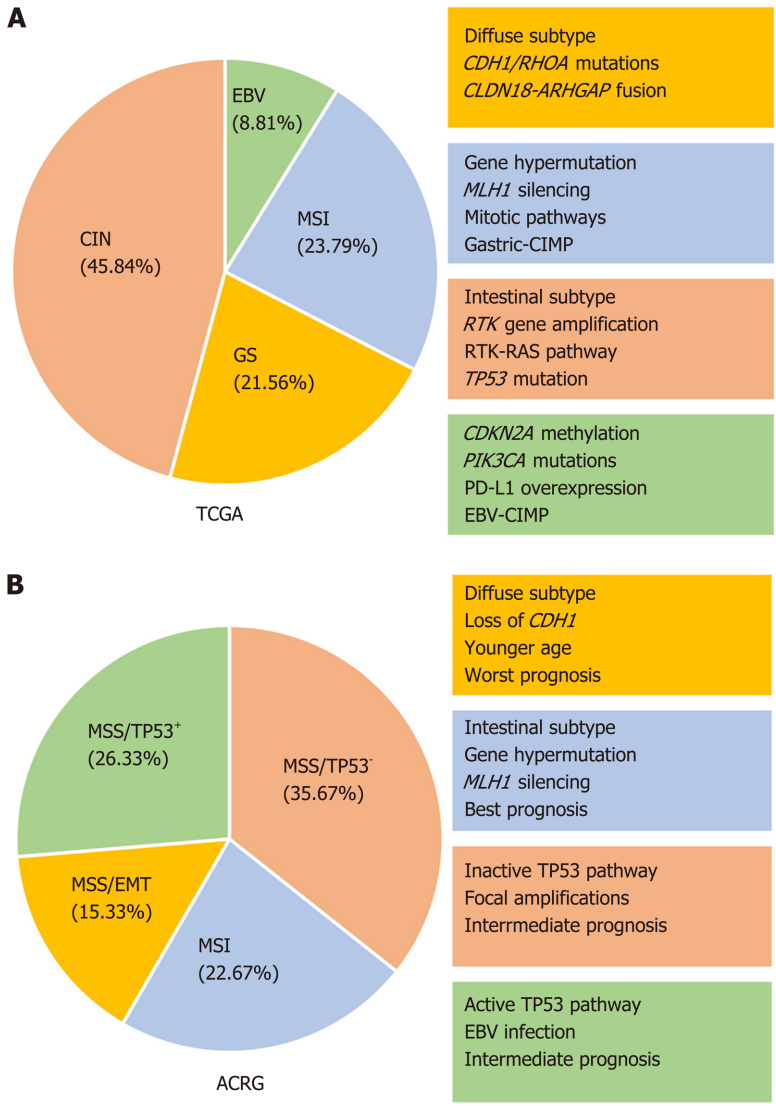

Figure 1 The cancer genome atlas and the Asian cancer research group molecular classification of gastric cancer. EBV: Epstein-Barr; CIN: Chromosomal instability; MSI: Microsatellite instability; GS: Genomically stable tumours; MSS/EMT: Microsatellite unstable type, epithelial to mesenchymal-like type.

anti-cancer treatment for their metastatic disease. As a result, all cases of advanced GC should be characterized for HER2 status. HER2 is a member of the epidermal growth factor receptors (EGFRs) family and is involved in transmembrane signaling, and overexpression/activation leads to increased cell proliferation, growth and survival[27]. HER2 overexpression or/and amplification is found in approximately 20% of metastatic GC, although there are differences depending on tumor subtype, is more common in intestinal GC than diffuse GC, and more common in GEJC than distal GC[28].

The phase III, open-label, randomised controlled ToGA trial (Trastuzumab for GC) compared in a population of 594 previously untreated patients standard chemotherapy (six courses of cisplatin plus either infusional fluorouracil or capecitabine) with and without trastuzumab until disease progression. All end points were improved with the addition of trastuzumab to chemotherapy, including objective response rate (ORR) (47.3% *vs* 34.5%), PFS (6.7 *vs* 5.5 mo; HR: 0.71; 95%IC: 0.59-0.85; $P < 0.0002$), and at a median follow-up of 17 to 19 mo, median OS was significantly better with trastuzumab (13.8 *vs* 11.1 mo) (HR: 0.74; 95%CI: 0.60-0.91; $P = 0.0046$). The inclusion criteria of the ToGA trial were a 3+ HER2 immunohistochemical (IHC) overexpression or the presence of *HER2* gene amplification as assessed by fluorescent in situ hybridization (FISH), irrespective of IHC score[29].

Despite the demonstrated efficacy of numerous chemotherapy options, only 40% of patients who progressed to first-line chemotherapy are susceptible to a second-line chemotherapy on progression[30]. In this setting, ramucirumab, a fully human monoclonal antibody VEGFR-2 antagonist, is the only molecular-targeted drug with a

confirmed, although modest, survival benefit.

The activity of ramucirumab, in second-line treatment of GC was investigated by the phase III REGARD trial (Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma), a randomized, double-blind, placebo-controlled study. In the REGARD trial 355 patients with previously treated advanced GC or GEJC adenocarcinomas were randomized to best supportive care plus either ramucirumab or placebo. Median OS was 5.2 mo in the ramucirumab arm and 3.8 mo in the placebo arm (HR: 0.78; 95%CI: 0.603-0.998; P = 0.047)[31]. However, the RAINBOW trial (Ramucirumab plus paclitaxel *vs* placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma) was the landmark study that demonstrated the benefit of ramucirumab in second line setting in combination with chemotherapy, which compared weekly paclitaxel (80 mg/m^2 on days 1, 8, and 15 of each 28-day cycle) plus ramucirumab (8 mg/kg IV every two wk) or placebo in 665 patients. Median OS and PFS were significantly longer in patients treated with ramicurumab than in the placebo-plus-paclitaxel group (median OS: 9.6 *vs* 7.4 mo, HR: 0.81, 95%CI: 0.678-0.962, P = 0.017 and median PFS 4.4 *vs* 2.9 mo, HR: 0.635, 95%CI: 0.539-0.752, $P \leq 0.001$, respectively)[32].

Largely based on this trial results, ramucirumab plus paclitaxel is currently the preferred choice for second-line therapy. More recently, the phase III TAGS study (Trifluridine/tipiracil *vs* placebo in patients with heavily pretreated metastatic GC) proved that trifluridine/tipiracil is an effective treatment option for patients with heavily pretreated metastatic GC. The study demonstrated a 31% reduction in risk of death and a 2.1-month improvement in median OS in treated patients[33].

HER2: PRIMARY AND ACQUIRED RESISTANCE

The anti-HER2 monoclonal antibody trastuzumab plus standard chemotherapy have significantly improved response rate and survival outcomes in primary GC and GEJC displaying HER2 overexpression/amplification. Unfortunately, about 50% of patients did not respond to the combination treatment suggesting the existence of a primary resistance[29]. At same time, acquired resistance usually limits the duration of response to this treatment.

Genomic alterations of the RTK pathway such as *EGFR*, *FGFR2*, *MET*, and *KRAS* amplification may be responsible for primary resistance to HER2-targeting drugs[14]. Recently, amplifications of cell-cycle–related genes such as *CCNE1* and *CDK6*, *PI3K* mutations, and amplification of *MET* have shown to confer resistance to anti-HER2 agents in vitro *HER2*–amplified cell-line models[34]. Although uncommon, other rare alterations in RTK pathways such as *ALK*, *ROS1*, *NTRK1/2/3* and *RET* fusion could be correlated with primary resistance to trastuzumab[35-37]. To confirm these data, a recent study investigated a panel of genomic alterations including mutations in the *EGFR / MET / KRAS / PI3K / PTEN* genes and amplifications in *EGFR / MET / KRAS* in 37 patients treated with trastuzumab (17 responders and 20 patients with primary resistance). Interestingly, panel alterations were significantly more frequent in resistant (11 of 20, 55%) as compared with sensitive patients and in HER2 IHC 2+ than 3+ tumors. Patients with no alteration had a significantly longer median PFS and OS[38].

Mechanisms of acquired resistance to anti-HER2 treatment in GC are largely unknown. Pietrantonio *et al*[39] have analyzed 22 matched tumor samples taken at baseline and post-progression in patients receiving chemotherapy and trastuzumab for advanced HER2 IHC 3+ or 2+ GC. Loss of HER2 was identified as a mechanism of resistance in 32% of samples and the probability of loss of HER2 positivity was significantly higher in patients with baseline IHC score 2+ *vs* 3+. Similarly, loss of HER2 and frequent secondary alterations in the RTK/RAS/PI3K pathway in HER2 positive adenocarcinoma have been observed in patients treated with trastuzumab[40].

In a recent study evaluating capecitabine and oxaliplatin as first line neoadjuvant therapy in patients with previously untreated, HER2-positive GC, the analysis of circulating free DNA (cfDNA) at disease progression demonstrated the emergences of genomic aberrations such as *MYC*, *EGFR*, *FGFR2* and *MET* amplifications[41]. However, none of these biomarkers is evaluated in the current clinical practice.

Other anti-HER2 strategies have failed to demonstrate a clinical benefit in second line HER2 positive GC. In the GATSBY trial (Trastuzumab emtansine *vs* taxane use for previously treated HER2-positive locally advanced or metastatic gastric or gastro-oesophageal junction adenocarcinoma) the antibody-drug conjugate consisting of the monoclonal antibody Trastuzumab linked to microtubule inhibitor emtansine (T-DM1) compared to taxans, failed to prolong survival in patients with previously

treated HER2-positive advanced GC[42]. In the phase III randomized TyTAN trial (Lapatinib plus paclitaxel *vs* paclitaxel alone in the second-line treatment of HER2-amplified advanced GC in Asian populations) comparing the efficacy and safety of the tyrosine kinase inhibitor of EGFR and HER2 Lapatinib plus paclitaxel with paclitaxel alone, the combination treatment demonstrated activity in the second-line but did not significantly improve OS in the intent-to-treat population (ITT)[43]. Moreover, in a recent trial comparing weekly paclitaxel alone with weekly paclitaxel plus Trastuzumab in patients with HER2-positive advanced GC/GEJC progressing during Trastuzumab-containing therapy, Trastuzumab beyond progression strategy did not improve PFS[44].

Results from ongoing phase III (NCT01774786) and phase II (NCT01522768) clinical trials of Pertuzumab and Afatinib respectively, will hopefully contribute to the unmet need in this setting of patients whose therapeutic options still remain limited (Table 1).

RTK/RAS - TARGET THERAPIES

CIN tumors are frequently characterized by the presence of activation of the RTK/RAS pathway and *EGFR*, *HER2*, *HER3*, *JAK2*, *MET*, *FGFR2*, *PIK3CA* and *KRAS/NRAS* amplification[14]. Other works have reported that at least one third of GC patients present alterations of the RTK/RAS pathway and may be potentially treatable by directed therapies[45]. Unfortunately, most of phase II and III trials evaluating RTK/RAS target therapies failed to demonstrate activity in metastatic GC.

The *EGFR* gene is amplified in the 5% and EGFR is overexpressed in more than 30% of GC[14,46]. Both anti-EGFR drug cetuximab and panitumumab have been tested in two phase III trial. In the EXPAND trial (Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced GC), the addition of the chimeric monoclonal antibody cetuximab to capecitabine-cisplatin provided no additional benefit in terms of PFS to chemotherapy alone in the first-line treatment of advanced GC (HR: 1.09; 95%CI: 0.92-1.29; P = 0.32)[47]. The REAL 3 trial (Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer), with 553 patients randomized to receive epirubicin, oxaliplatin, and capecitabine (EOC) plus the human monoclonal antibody panitumumab or EOC alone, failed to show a benefit in OS of the combination therapy compared with the only chemotherapy (HR: 1.37; 95%CI: 1.07-1.76; P = 0.013)[48]. However, none of these studies have selected patients on the basis of *EGFR* overexpression/amplification. In metastatic colorectal cancer, *RAS* mutations are a negative predictive biomarkers of response to anti-EGFR therapy but can be detected only in about 3% of GC and GEJC.

Several works have reported that EGFR expression, *EGFR* gene copy number, or expression of other EGFR ligands such as epiregulin and amphiregulin, might be potential markers for efficacy of anti-EGFR target therapies[49-51]. However, in the EXPAND trial, no substantial differences between the treatment groups for PFS or OS according to EGFR immunohistochemistry score was noted[47]. Results from a phase III trial comparing the efficacy of nimotuzumab, a recombinant humanized anti-EGFR antibody, and irinotecan on irinotecan alone in patients with EGFR overexpressed advanced GC/GEJC are expected (ENRICH study, NCT01813253, Table 1).

The tyrosine kinase receptor c-MET and its own ligand, hepatocyte growth factor (HGF), have been investigated as potential target in advanced GC. In GC, alteration of the MET/HGF pathway is related to a more aggressive disease and poor prognosis, with MET activation stimulating tumor invasiveness[52,53]. Onartuzumab, a monovalent monoclonal antibody binding with the extracellular of MET, has been tested in a phase III trial of 562 patients randomized to receive onartuzumab plus FOLFOX6 *vs* placebo plus mFOLFOX6 in patients with metastatic HER2-negative and MET-positive GEC. However, the addition of onartuzumab to mFOLFOX6 did not attain significant differences in OS or PFS compared with placebo plus mFOLFOX6 in ITT (OS HR: 0.82; 95%CI: 0.59-1.15; P = 0.24; PFS HR: 0.90; 95%CI: 0.71-1.16; P = 0.43) or MET 2+/3+ populations (OS HR: 0.64; 95%CI: 0.40-1.03; P = 0.06; PFS HR: 0.79; 95%CI: 0.54-1.15; P = 0.22)[54]. The RILOMET phase III trial (Rilotumumab plus epirubicin, cisplatin, and capecitabine as first-line therapy in advanced MET-positive gastric or gastro-oesophageal junction cancer), evaluating the fully human monoclonal antibody anti-MET Rilotumumab plus epirubicin, cisplatin, and capecitabine or placebo plus epirubicin, cisplatin, and capecitabine as first line in advanced GC, was ceased subsequently the finding by an independent data monitoring committee of a higher number of deaths in the rilotumumab group compared with the placebo group[55].

Table 1 Ongoing phase II/III target trials in advanced gastric cancer

Study	Line	Control arm	Experimental arm	Target	NCT number
JACOB	1st	Placebo + Trastuzumab + Chemotherapy	Pertuzumab + Trastuzumab + Chemotherapy	HER2	NCT01774786
ID NUMBER:11-166	2nd	-	Afatinib + Paclitaxel	HER2	NCT01522768
NIEGA	2nd	-	Irinotecan + Nimotuzumab	EGFR	NCT03400592
ENRICH	2nd	Irinotecan	Irinotecan + Nimotuzumab	EGFR	NCT01813253
CheckMate-649	1st	Oxaliplatin + Fluoropyrimidine	- Nivolumab + Oxaliplatin + Fluoropyrimidine - Ipilimumab + Nivolumab	PD-1, CTLA-4	NCT02872116
ATTRACTION-4	1st	Placebo + Oxaliplatin + S-1/Capecitabine	Oxaliplatin + S-1/Capecitabine + Nivolumab	PD-1	NCT02746796
JAVELIN Gastric 100	1st	Maintenance 1st line	Avelumab	PD-L1	NCT02625610
KEYNOTE-062	1st	Platin/fluoropyrimidine	- Pembrolizumab - Pembrolizumab + Platin/fluoropyrimidine	PD-1	NCT02494583
SPOTLIGHT	1st	Oxaliplatin + Fluoropyrimidine	Zolbetuximab + Oxaliplatin + Fluoropyrimidine	CLDN18.2	NCT03504397
ILUSTRO	1st/3rd	-	- Zolbetuximab monotherapy, 3rd line - Zolbetuximab + FOLFOX, 1st line	CLDN18.2	NCT03505320
GLOW	1st	Oxaliplatin + Capecitabine	Zolbetuximab + Oxaliplatin + Capecitabine	CLDN18.2	NCT03653507
ANGEL	3rd	BSC	Apatinib	VEGFR-2	NCT03042611
INTEGRATE II	3rd	Placebo	Regorafenib	VEGFR1-3, FGFR, PDGFR-β RAF, RET and KIT	NCT02773524

JACOB: A Study of Pertuzumab in Combination With Trastuzumab and Chemotherapy in Participants With Human Epidermal Growth Factor Receptor 2 Positive Metastatic Gastroesophageal Junction or Gastric Cancer; ID NUMBER:11-1669: Afatinib and Paclitaxel in Patients With Advanced HER2-Positive Trastuzumab-Refractory Advanced Esophagogastric Cancer; NIEGA: Study of Nimotuzumab and Irinotecan as Second Line With Recurrent or Metastatic Gastric Adenocarcinoma; ENRICH: Study of Nimotuzumab and Irinotecan as Second Line With Advanced or Recurrect Gastric and Gastroesophageal Junction Cancer; CheckMate649: Efficacy Study of Nivolumab Plus Ipilimumab or Nivolumab Plus Chemotherapy Against Chemotherapy in Stomach Cancer or Stomach/Esophagus Junction Cancer; ATTRACTION-4: Study of ONO-4538 in Gastric Cancer; JAVELIN: Gastric 100Avelumab in First-Line Maintenance Gastric Cancer; KEYNOTE-062: Study of Pembrolizumab as First-Line Monotherapy and Combination Therapy for Treatment of Advanced Gastric or Gastroesophageal Junction Adenocarcinoma; SPOTLIGHT: A Phase 3 Efficacy, Safety and Tolerability Study of Zolbetuximab Plus mFOLFOX6 Chemotherapy Compared to Placebo Plus mFOLFOX6 as Treatment for Gastric and Gastroesophageal Junction Cancer; ILUSTRO: A Study to Assess the Antitumor Activity, Safety, Pharmacokinetics and Biomarkers of Zolbetuximab in Participants With Claudin 18.2 Positive, Metastatic or Advanced Unresectable Gastric and Gastroesophageal Junction Adenocarcinoma; GLOW: A Study of Zolbetuximab Plus CAPOX Compared With Placebo Plus CAPOX as First-line Treatment of Subjects With Claudin 18.2-Positive, HER2-Negative, Locally Advanced Unresectable or Metastatic Gastric or Gastroesophageal Junction Adenocarcinoma; ANGEL: Efficacy and Safety Trial of Apatinib Plus Best Supportive Care Compared to Placebo Plus Best Supportive Care in Patients With Gastric Cancer; INTEGRATEII: A Study of Regorafenib in Refractory Advanced Gastro-Oesophageal Cancer, Best supportive care; HER2: Human epidermal growth factor receptor 2; EGFR: Epidermal growth factor receptor; PD-L1: Programmed death ligand 1; PD-1: Programmed cell death protein 1; CTLA-4: Cytotoxic T-lymphocyte-associated antigen 4; CLDN18.2: Claudine-18.2; VEGFR1-3: Vascular endothelial growth factor receptor 1-3; FGFR: Fibroblastic growth factor receptor; PDGFR-β: Platelet-derived growth factor receptor beta; RAF: Serine/threonine-specific protein kinases RAF; RET: Rearranged during transfection; KIT: Tyrosine-protein kinase Kit.

Approximately 5%-10% of GCs present an *fibroblast growth factor receptor-2* (*FGFR2*) gene amplification, which appears to confer poor prognosis[56-58]. The selective FGFR-1, 2, 3 tyrosine kinase inhibitor AZD4547 showed potent activity in preclinical models[59]. The randomized phase II SHINE study (Efficacy and Safety of AZD4547 *vs* Paclitaxel in Patients With Advanced Gastric or Gastro-oesophageal Cancer) comparing

AZD4547 *vs* paclitaxel as second-line treatment in patients with advanced GC displaying *FGFR2* polysomy or gene amplification did not demonstrated a PFS improvement in the experimental arm (1.8mo with AZD4547 *vs* 3.5mo with paclitaxel; HR: 1.57; 80%CI: 1.12-2.21; $P = 0.9581$)[60].

IMMUNOTHERAPY

GCs/GEJCs are associated with immune system evasion and overexpression of immune checkpoint proteins including the programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2) expressed on the surface of tumor and immune cells. An high expression of PD-L1 has been reported in both Western and Asian GC/GEJC cohorts and has been associated with an elevated tumor mutational burden (TMB) and specific subtypes of adenocarcinomas[61,62]. The binding of PD1, a protein of CD28 family expressed on T cells functioning as a negative costimulatory receptor, and its ligands-PD-L1 and PD-L2, can inhibit cytotoxic T-cell responses, allowing tumor cells to evade immune surveillance. Checkpoint inhibitors such as antibodies anti PD1 (pembrolizumab, nivolumab) and PD-L1 (atezolizumab, avelumab, durvalumab) and inhibitors of cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4, like ipilimumab) have demonstrated to antagonize this immune tolerance, which results in an enhanced antitumor effect. In the last years, checkpoint inhibitors have shown activity in several solid tumors and have received approval for a number of clinical indications including advanced melanoma, renal cell carcinoma and non-small lung cancer (NSCLC)[63].

Since their introduction in the treatment scenario, lots of efforts have been undertaken to establish predictive biomarkers of response to these novel agents. Combined data from disease-specific trials with the humanized IgG4 monoclonal antibody pembrolizumab, demonstrated that tumors with a large number of somatic mutations due to high microsatellite instability (MSI-H) or mismatch repair deficiency (dMMR) are susceptible and can benefit to immune checkpoint blockade. On these findings, in 2017 FDA decided to accelerate the approval to Pembrolizumab for patients with unresectable or metastatic solid tumours with positive dMMR or MSI-H biomarkers[64]. Other studies have shown that PD-L1 expression on the membranes of tumor cells or tumor-infiltrating immune lymphocytes (TILs) was associated with a better OS in certain types of tumours treated with checkpoint inhibitors. However, there is currently no consensus on the role of PD-L1 expression as prognostic and predictive biomarker in advanced GC[65].

In GC, checkpoint inhibitors have been firstly assessed in the salvage setting showing a rather wide range of response rate (11.6%-22%)[66,67]. In the phase III Asian ATTRACTION-2 trial (Nivolumab in patients with advanced gastric or gastro-oesophageal junction cancer refractory to, or intolerant of, at least two previous chemotherapy regimens) 493 patients with refractory GC to two or previous chemotherapy regimens were randomized to receive nivolumab ($n = 330$) or placebo ($n = 163$) resulting in a median OS of 5.26 mo (95%CI: 4.60-6.37) in the nivolumab group and 4.14 mo (3.42-4.86) in the placebo group (HR: 0.63; 95%CI: 0.51-0.78; $P < 0.0001$). The OS rates of nivolumab and placebo were 27.3% and 11.6% at 12 mo, and 10.6% and 3.2% at 24 mo, respectively. Based on these results, nivolumab was granted accelerated approval in Japan, South Korea and Taiwan for the treatment of advanced GC progressed to standard chemotherapy[68].

Moreover, Pembrolizumab has recently received accelerated approval by FDA considering the promising results of the KEYNOTE-059 trial (Safety and Efficacy of Pembrolizumab Monotherapy in Patients with Previously Treated Advanced Gastric and Gastroesophageal Junction Cancer). In this phase II, single arm study, 259 patients with advanced GC or GEJC whose disease progressed to two or more lines of therapy, received pembrolizumab every 3 wk achieving an objective response rate (ORR) of 11,6 % (95%CI: 8.0%-16.1%; $n = 30/259$) with complete response in 2.3% (95%CI: 0.9%-5%; $n = 6/259$) and manageable safety. Interestingly, patients with PD-L1 positive tumors (PD-L1 combined positive score ≥ 1) had an ORR of 22.7% (95%CI: 13.8-33.8) and patients PD-L1-negative had an ORR of only 8.6% (95%CI: 2.9-19.0). Excluding MSI-H tumors (ORR of 57%, 4 of 7 patients) from that group, the percentage of response to pembrolizumab decreased to 13.3% in PD-L1 positive microsatellite stable (MSS) (or MSI status not available) patients, and 9% (15 of 167) of MSS patients independently of PD-L1 status responded, confirming the importance of the microsatellite status as marker of response to checkpoint inhibitors[66].

Despite the initial enthusiasm, some randomized phase III trial reported negative outcomes with checkpoint inhibitors when compared to chemotherapy. The KEYNOTE-061 phase III trial (Pembrolizumab *vs* paclitaxel for previously treated,

advanced gastric or gastro-oesophageal junction cancer) comparing pembrolizumab *vs* chemotherapy with weekly paclitaxel as second line treatment in patients with GC or GEJC with PD-L1 positivity in at least 1 % of tumor cells, failed to improve OS and PFS[69]. Similarly, the randomized, phase III Javelin Gastric 300 trial comparing the anti-PD-L1 IgG1 monoclonal antibody Avelumab *vs* chemotherapy as third line therapy in 379 patients with advanced GC/GEJC, did not meet its primary endpoint of improving OS or the secondary end points of PFS[70].

In the TCGA study an high level of intra- or peritumoral immune cell infiltration and frequent amplification of the *CD274* gene (which encodes PD-L1) and the *PDCD1LG2* gene (which encodes PD-L2) in the EBV-positive subgroup GC was found[14]. Furthermore, subsequent studies confirmed remarkable PD-L1 expression both in cancer and immune cells in EBV positive GCs[71]. Consistent with these findings, a prospective phase II Korean clinical trial of pembrolizumab with whole exome and RNA sequencing in pre and post biopsy specimens was performed to better define those patient who may benefit from pembrolizumab. Among 61 patients with advanced GC that received pembrolizumab as a second or greater line of treatment, those with MSI-H and EBV positive tumours, which are mutually exclusive, showed dramatic responses to pembrolizumab with ORR of 85.7% (6/7) in the MSI-H group and of 100% (6/6) EBV positive GCs. In addition for the 55 patients for whom PD-L1 combined positive score positivity (cut off value ≥ 1) was available, ORR was significantly higher for PD-L1 positive (n = 28) tumors when compared to PD-L1 negative (n = 27) GCs (50.0% *vs* 0.0%, P < 0.001)[72]. Although this study have provided the first clinical evidence of high activity of pembrolizumab EBV positive GCs, the percentage of EBV positive or MSI-H GCs was higher in this patient cohort compared to Western cohorts. This can be explained at least in part with the different regional risk of acquiring EBV associated GCs that ranges from 1.3%-30.9% (average of 10% worldwide) with the highest risk in Far East Asia, which also presents the highest incidence of GCs[73].

In order to optimize treatment strategies with checkpoint inhibitors, a number of ongoing trials are evaluating these agents in the first line setting (NCT02872116, NCT02746796, NCT02625610, NCT02494583, Table 1). Novel predictive biomarker are needed to select patient subgroups most likely to benefit from checkpoint inhibitors. Recently, Sundar *et al*. reported that epigenomic promoter alterations occur in a substantial proportion of metastatic GCs and cause reduced expression immunogenic peptides, which allow immune evasion and remarkable resistance to anti-PD1 immune checkpoint inhibition[74].

CLAUDIN 18.2

Claudins are main components of tight junctions in epithelial cellular sheets. Distinct claudins isoforms have been identified in different organs and their altered function has been discovered to be associated to the cancerogenesis of respective tissues[75,76]. Claudin 1-5, 7-12,16 and 18 proteins are expressed in healthy gastric tissue[77]. In particular the isoform 2 of the tight junction molecule Claudine-18 (CLDN18.2) is strictly confined to differentiated gastric epithelial cells where controls the paracellular permeability to Na^+ and H^+. In a significant percentage of primary GCs and metastases, the cell polarity perturbations lead to exposure of CLDN18.2 on the surface of GC cells so that it is available for monoclonal antibody binding[78]. CLDN18.2 is not exclusive of GC and is broadly expressed in various cancer types including biliary duct, pancreatic, ovarian cancer and NSCLC. A recent work have analyzed 286 GC/GEJC tissue samples from North America, Asia and Europe, demonstrating that 30% of samples (n = 86/286) presented high expression CLDN18.2 (moderate-to-strong CLDN18.2 membrane staining in ≥ 75% of tumor cells) with limited overlap with HER2[79]. These biological findings suggested that CLDN18.2 could be targetable and led to the further development of monoclonal antibodies against this protein. Zolbetuximab (IMAB362) is an anti-CLDN18.2 antibody that binds GC cell lines with high relative affinity and selectivity, then mediates a lysis of CLDN18.2-positive cells through antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). In the phase II FAST trial a total of 161 patients were randomized to receive zolbetuximab plus epirubicine and oxaliplatin (EOX) or EOX alone. Median PFS was significantly higher with zolbetuximab + EOX (7.5 mo) *vs* EOX alone (5.3 mo; P < 0.0005; HR: 0.44, 95%CI: 0.29-0.67) and median OS (13 *vs* 8.4 mo; P = 0.0008; HR: 0.56, 95%CI: 0.40-0.79) and ORR (39 *vs* 25%; P = 0.022) were also demonstrated to be longer with zolbetuximab + EOX *vs* EOX alone with an increased efficacy in patients with high CLDN18.2 expression[80]. Consistent with these results, several trials are investigating zolbetuximab in different

setting (NCT03504397, NCT03504397, NCT03653507, Table 1).

ANGIOGENIC AND STROMAL INHIBITORS

Based on the positive results of the REGARD and RAINBOW trial, other agents were assessed for angiogenic inhibition in GC. The VEGFR-2 tyrosine kinase inhibitor apatinib was tested in a phase II trial of patients with advanced GC refractory to two or more lines of prior chemotherapy, showing compared to placebo, prolonged OS (6.5 mo; 95%CI: 4.8 -7.6 *vs* 4.7 mo; 95%CI: 3.6-5.4; $P = 0.0149$; HR: 0.709; 95%CI: 0.537-0.937; $P = 0.0156$) and PFS (2.6 mo; 95%CI: 2.0-2.9 *vs* 1.8 mo; 95%CI: 1.4-1.9; $P < 0.001$; HR: 0.444; 95%CI: 0.331-0.595; $P < 0.001$)[81]. The ongoing ANGEL phase III trial (Efficacy and Safety Trial of Apatinib Plus Best Supportive Care Compared to Placebo Plus Best Supportive Care in Patients With Gastric Cancer) is evaluating the clinical benefit and safety of apatinib plus Best Supportive Care (BSC) in comparison to placebo plus BSC in patients who failed to at least two prior lines of standard chemotherapies (NCT03042611, Table 1). Other phase III trials are assessing the efficacy of apatinib as maintenance treatment after first line induction therapy (NCT03598348, NCT02510469, NCT02509806). Regorafenib is an oral multi-kinase inhibitor which targets angiogenic (VEGFR-1 and -2, tie-2), stromal (PDGF-β) and oncogenic RTK, largely used in metastatic colorectal cancer and GIST. In the INTEGRATE phase II study (Regorafenib for the treatment of advanced GC) patients with previously treated GC had statistically significantly improved PFS when treated with regorafenib compared to placebo [2.6 *vs* 0.9 mo (HR: 0.40; 95%CI: 0.28-0.59; stratified log-rank: $P < 0.001$)][82]. Consistent with these results, regorafenib is currently evaluated in the INTEGRATE II phase III trial (NCT02773524, Table 1). Bevacizumab is a recombinant humanized monoclonal antibody that blocks angiogenesis by inhibiting VEGF-A. The AVAGAST and AVATAR trials, comparing the VEGF-antibody bevacizumab plus cisplatin/capecitabine to chemotherapy alone in different populations, failed to show significant benefit in OS[83,84]. Subgroup analysis of the AVAGAST trial showed that non-Asian patients were more likely to benefit from an anti-angiogenic therapy than Asian patients, although in the overall study population, this effect was not observed. Despite the encouraging results in the second line setting, in the recent phase III trial RAINFALL (Ramucirumab with cisplatin and fluoropyrimidine as first-line therapy in patients with metastatic gastric or junctional adenocarcinoma) that randomized patients to receive ramucirumab plus fluoropyrimidine and cisplatin or placebo plus fluoropyrimidine and cisplatin as first-line treatment, the addiction of ramucirumab to chemotherapy did not demonstrated a statistical significant advantage in PFS (HR: 0.961, 95%CI: 0.768-1.203, $P = 0.74$) and OS [HR: 0.962, 95%CI: 0.801-1.156, $P = 0.6757$; median OS 11.2 mo (9.9-11.9) in the ramucirumab group *vs* 10.7 mo (9.5-11.9) in the placebo group][85]. Other studies have investigated innovative approach to target the tumor microenvironment. A phase I/Ib study found that the addition of andecaliximab, a monoclonal antibody that inhibits matrix metalloproteinase 9, to FOLFOX showed activity in GC and GEJC. Unfortunately the phase III GAMMA-1 trial (A phase III, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of andecaliximab combined with mFOLFOX6 as first-line treatment in patients with advanced gastric or gastroesophageal junction adenocarcinoma) comparing FOLFOX6 plus andecaliximab or mFOLFOX6 plus placebo showed a median OS of 12.5 *vs* 11.8 mo in the andecaliximab *vs* placebo treatment groups, respectively (HR: 0.93, two-sided: $P = 0.56$) and a median PFS of 7.5 mo in the andecaliximab group *vs* 7.1 mo in the placebo group (HR: 0.94, two-sided: $P = 0.10$)[86].

CONCLUSION

Recent high-throughput molecular profiling studies have provided a deeper understanding of the multiple genomic and epigenetic landscape of this complex and heterogeneous disease. New gene mutations, chromosomal aberrations, transcriptional and epigenetic alterations have been described with potentially implications for the development of personalized treatment options. However, at present, few target therapies are still available for metastatic GC.

Researches are focusing on the comprehension of primary and acquired mechanisms of resistance to anti-HER2 drugs. Moreover the targeting of other RTKs such as EGFR, MET or FGFR by TKIs or monoclonal antibodies failed to demonstrate a clinical benefit in GC. However, an appropriate molecular selection have not been conducted in many target driven clinical trials and retrospective analyses of these

studies have provided a potential benefit from RTK-inhibitors in molecularly selected subgroups.

It has to be noted that an excessive GC tumor heterogeneity and evolution complicates the efficacy of target strategies. Recent studies showed a significant discrepancy in genomic alterations within the primary tumor and between the primary tumor and disseminated disease and the potential use of plasma-based circulating-tumor DNA (cfDNA) to enhance selection of therapy in GC[41,87].

Based on the promising results of clinical trials of patients with pretreated advanced GC, pembrolizumab and nivolumab were granted accelerated approval in in the United States and in some Asian countries respectively. In contrast, none of the current checkpoint inhibitors have been approved by the European Medicines Agency (EMA). As demonstrated in other solid tumors, GC with MSI-H or dMMR is more likely to respond to checkpoint inhibitors. EBV positive GCs seem to benefit significantly from these drugs, while the role of PD-L1 expression as prognostic and predictive biomarker of response to checkpoint inhibitors has not confirmed in all the studies. In addition, epigenomic promoter alterations have been recently described as a novel potential mechanism of resistance to checkpoint inhibitors in a substantial proportion of GC. The anti-CLDN18.2 antibody zolbetuximab has shown promising results and it is currently investigated in different ongoing trials. As regard angiogenesis, in addition to ramucirumab, other antiangiogenic agents including apatinib and regorafenib are currently under investigation.

In conclusion, remarkable advances in the molecular characterization of GC have expanded our knowledge and paved the way to novel treatment options that will hopefully improve the survival outcomes of patients with metastatic GC.

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