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FIELD OF VISION

Molecular classifications of gastric cancers: Novel insights and possible future applications

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Abstract

Despite some notable advances in the systemic management of gastric cancer (GC), the prognosis of patients with advanced disease remains overall poor and their chance of cure is anecdotic. In a molecularly selected population, a median overall survival of 13.8 mo has been reached with the use of human epidermal growth factor 2 (HER2) inhibitors in combination with chemotherapy, which has soon after become the standard of care for patients with HER2-overexpressing GC. Moreover, oncologists have recognized the clinical utility of conceiving cancers as a collection of different molecularly-driven entities rather than a single disease. Several molecular drivers have been identified as having crucial roles in other tumors and new molecular classifications have been recently proposed for gastric cancer as well. Not only these classifications allow the identification of different tumor subtypes with unique features, but also they serve as springboard for the development of different therapeutic strategies. Hopefully, the application of standard systemic chemotherapy, specific

targeted agents, immunotherapy or even surgery in specific cancer subgroups will help maximizing treatment outcomes and will avoid treating patients with minimal chance to respond, therefore diluting the average benefit. In this review, we aim at elucidating the aspects of GC molecular subtypes, and the possible future applications of such molecular analyses.

Key words: Molecular biology; Immunotherapy; Gastric cancer; Classification; Targeted therapy

Core tip: TCGA individuates four molecular subtypes: Chromosomal instability, microsatellite instability, genomically stable and Epstein-Barr virus positive tumors. Asian Cancer Research Group classification partially overlaps with the previous one. Although not prospectively validated, these novel classifications suggest that different subtypes of gastric cancer might be treated with specific therapeutic strategies in the near future.

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INTRODUCTION

Gastric cancer (GC) is among the most common malignancies worldwide and the second leading cause of cancer related deaths[1]. In fact, it represents the fifth most commonly diagnosed cancer (6.8% of oncologic diagnoses) resulting in an annual estimated incidence of 18 cases out of 100000 individuals among men and 9 out of 100000 for women[2].

The mainstay of first-line therapy for GC is still represented by a chemotherapy backbone composed by platinum compounds and fluoropyrimidines resulting in a median overall survival (OS) of about 11 mo. Still, the disappointing 5-year survival rate is estimated to be about 25%-30% and slightly higher for some Asian experience. Historically, many attempts have been made in order to re-classify gastric cancer with the aim of clustering some new subgroups that could have different prognostic and predictive value: Anatomical classification (Borrmann classification and Siewert and Stein classification), histological classification (WHO classification and Lauren's classification), and extent of disease (early gastric cancer *vs* advanced cancer).

The first effective molecular novelty came from the TOGA trial which demonstrated a significant improvement in OS with the addition of trastuzumab to chemotherapy when compared to chemotherapy alone in patients with HER2 overexpressing GCs (13.8 mo *vs* 11 mo, respectively; $P = 0.046$)[3]. Another clue to the "heterogeneity theory" comes from the observation that Asian patients demonstrate different pattern of disease and outcomes if compared to the Caucasian western population included in the largest trials.

Nowadays, with mounting biological information available, almost every solid cancer type is considered as a "collection" of multiple very molecularly heterogeneous diseases. Very important advances have been made in the molecular classification of breast cancers[4], lung tumors (by the identification of some tyrosine-kinase-inhibitor targetable subtypes), colorectal adenocarcinomas (predictive and prognostic classes sorted by mutations in *RAS* and *BRAF* genes), and malignant melanoma (identification of *BRAF* codon 600 mutation).

Nevertheless, the poor anatomical and molecular selections of GC patients entering clinical trials have potentially limited the effect of many therapeutic agents including chemotherapy, antiangiogenic drugs and the newly tested immune-modulators. In fact, the benefit of those drugs may have been diluted when tested in the overall population. Recently something has changed the way of thinking GC starting from the TCGA group publication appeared in 2014[5].

A more profound understanding of the molecular clustering of stomach cancer could give us the chance to obtain new insights into prognostic and predictive categorization of this cancer and could definitely provide the scientific knowledge for developing modernly conceived clinical trials that could maximize the effect of novel agents in the proper patient population, avoiding the use of costly drugs in non-stratified populations.

Finally, the aim of this review is to give a general picture of the current knowledge of the emerging molecular classification of GC and to explore the new possibilities connected to the latest discoveries made on the extreme heterogeneity of this disease.

THE IMPORTANCE AND LIMITATIONS OF MOLECULAR CLASSIFICATIONS

The first attempt to generate a comprehensive molecular classification for GC was made in 2013 by Singapore Researchers[6]. They identified three main types of gastric cancer, namely proliferative (characterized by high genomic instability and *TP53* mutation), metabolic (more sensitive to 5-FU therapy) and mesenchymal (stem cell-like tumors sensitive to PIK3CA-mTOR pathway inhibitors), based on genome expression. Soon after the TCGA research group published a classification dividing GCs into four main subgroups clustered on the basis of six different molecular biology approaches: Copy number variation (CNV) analysis, exome sequencing analysis, DNA methylation profile, mRNA sequencing, micro-RNA (miRNA) sequencing and reverse phase protein array[5]. The result

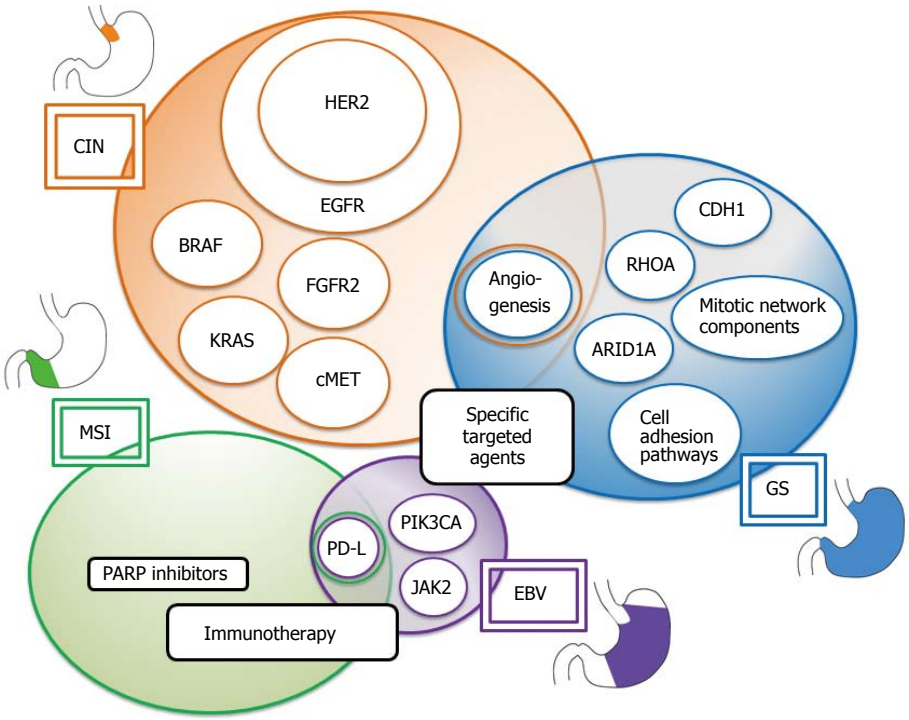

Figure 1 Four molecular subtypes of gastric cancer (chromosomal instability, genomical stability, microsatellite instability, and Epstein-Barr virus) are represented. Particular anatomic distribution and prospective therapeutic strategies. The areas represent the epidemiologic extent of each of the subtypes. On the side of each subtype the anatomical distribution is displayed. CIN: Chromosomal instability; GS: Genomical stability; MSI: Microsatellite instability; EBV: Epstein-Barr virus.

is the subdivision of GC into four genomic subtypes: Epstein-Barr virus (EBV) positive cancers (9% of all gastric tumors with frequent *PIK3CA* mutation and PD-L1/PD-L2 overexpression), Microsatellite Instability tumors (MSI, representing 22% and hypermutated), chromosomal instability (CIN, 50%, predominantly junctional, *TP53* mutated with RTK-RAS activation, with a high rate of CNV) and Genomically Stable (GS, 20%, presenting mutation in motility and adhesion molecules). Specific TCGA molecular subtypes are represented in Figure 1.

In the meantime, the Asian Cancer Research Group (ACRG) too proposed a novel molecular classification[7], and the resulting taxonomy divided GCs into: Mesenchymal subgroup (MSS/EMT, characterized by hallmarks of epithelial-to-mesenchymal transition), Microsatellite Instability subgroup (MSI), Microsatellite Stable *TP53* positive (MSS/TP53$^+$, somehow overlapping with EBV type of TCGA classification) and Microsatellite Stable *TP53*-tumors (MSS/TP53$^-$, overlapping with CIN by TCGA).

These novel classifications create a new paradigm in the definition of cancer biology and allow the identification of relevant genomic subsets by using different techniques such as genomic screenings, functional studies and molecular or epigenetic characterization. However, some limitations should also be openly recognized. First, these classifications are based on a highly complex methodology and currently they should not be replicated in standard laboratories lacking in the uttermost technologies. Attempts towards simplification are ongoing although results may not fully capture the underpinning complexity of the disease. Second, these classifications lack of a prospective validation on a large scale, including patients with different ethnicity and age. Third, the two proposed classifications have more differences than similarities; in particular, they are different in terms of demographics, baseline molecular mechanisms, driver genes, and association with prognosis. Moreover, there are notable dissimilarities in the distribution of Lauren's diffuse subtype among the different subgroups. Since different molecular subgroups may be identified across a number of independent gene expression profile studies, a collaborative international effort is warranted to aggregate a consensus classification. Fourth, the follow-up of included patients is limited, factor that may decrease their prognostic power, and subgroups were evaluated on resected specimens, with different prevalence of subgroups between localized, locally advanced and advanced settings. Fifth, both classifications insist on epithelial cells, but none of them take into account the active, nonmalignant stromal cells. Actually, not only gene expression profiles deriving from stromal tissues may influence assignment to a specific molecular category, thus creating interpretative troubles[8], but also novel stromal-based distinctive signatures have been proposed and related to the predominant cancer phenotype[9].

GC WITH CHROMOSOMAL INSTABILITY

CIN subtype represents approximately 50% of GCs[10] and it mostly occurs in the esophagogastric junction (EGJ)/cardia. CIN GC is related to intestinal type histology,

to copy number gains of chromosomes 8q, 17q and 20q, while, gains at 12q and 13q are associated with diffuse GC[11]. Interestingly, CIN showed elevated frequency in the EGJ/cardia, as demonstrated in TCGA characterization (65%, $P = 0.012$). CIN is characterized by somatic mutations at cytogenetic level, particularly involving loci that control mitotic checkpoints, thus gatekeeper and caretaker genes implicated in carcinogenesis. CIN comprises both altered DNA copy number and structural abnormalities in some chromosomal regions. Those alterations could result in gain or loss of whole chromosomes[12] (aneuploidy), non-reciprocal translocations, amplifications, deletion or the loss of one allele with loss of heterozygosis. Altogether, CIN results in the loss or gain of function of some "key genes", including oncogenes and tumor suppressor genes that may be efficaciously targeted by specific inhibitor molecules[13]. Notably, CIN GC is enriched in mutations in *TP53* gene and receptor tyrosine kinases (RTKs), furthermore it shows amplifications of cell cycle genes (Cyclin E1, Cyclin D1, and Cyclin-dependent kinase 6)[14].

Evaluation of the biological characteristics among CIN cancers demonstrated that *TP53* mutations occurs in 71% of GCs[5]. Furthermore, CIN also display amplification in oncogene pathways such as RTK/RAS/MAPK signaling, including HER2, BRAF, epidermal growth factor (EGFR), MET, FGFR2, RAS[5,15].

A recent work reviewed the pathogenic and molecular similarities between gastric intestinal-type adenocarcinoma and esophageal adenocarcinoma (EAC)[16], suggesting that treatment of EAC should recall that of gastric adenocarcinoma rather than being similar to the approach used for upper esophageal cancers (mostly squamous). In fact, not only EAC may arise from progenitor cells deriving from the cardia of the stomach but also the majority of EAC express a chromosomal instability that closely resembles the one found in CIN GC. All these findings suggest both the need for better subtyping esophageal cancers and the opportunity of developing specific therapeutics strategies in this disease as well.

HER2

The proto-oncogene HER2 is a member of the EGF receptor family with tyrosine kinase activity. It is known that HER2 positivity may vary depending on the primary tumour location as well as on the histotype of gastric cancer. Indeed, *HER2* overexpression/amplification is detected in more than 30% of the tumours arising from the gastroesophageal junction whereas less than 20% of tumours in the gastric body are HER2-positive. In addition, intestinal and diffuse histotype display a rate of HER2 positivity of 34% and 6% respectively[17]. HER2 plays a key role in a large number of cellular processes, including cell differentiation, proliferation, motility and signal transduction. After the combination of chemotherapy and HER2 targeted therapy with trastuzumab had defined a new standard of care for HER2-positive metastatic GC[3,18,19], other HER2 inhibitors were tested.

Lapatinib, a multi-kinase inhibitor, was evaluated in two randomized phase Ⅲ trials enrolling GC patients with advanced disease. The LOGiC trial tested the efficacy of lapatinib in combination with capecitabine plus oxaliplatin given upfront. The addition of lapatinib did not significantly increase OS [12.2 mo *vs* 10.5 mo, hazard ratio (HR) 0.91, $P = 0.349$], although progression-free survival (PFS) was longer (6.0 mo *vs* 5.4 mo, HR 0.82, $P = 0.0381$) and objective response rate (ORR) was higher (53% *vs* 39%, $P = 0.0031$) in the lapatinib arm[19]. The TyTAN trial randomized 261 Asian patients to receive lapatinib plus paclitaxel or paclitaxel alone in second-line treatment. Disappointingly, no marked survival differences between treatment groups were noted: Median OS (11.0 mo *vs* 8.9 mo, $P = 0.1044$) and PFS (5.4 mo *vs* 4.4 mo, $P = 0.2441$). Overall, 15 patients (6%) had previously received trastuzumab, 8 in the lapatinib/paclitaxel arm and 7 in the paclitaxel alone arm[20].

JACOB, a large randomized phase Ⅲ trial designed to test the efficacy of pertuzumab in combination with trastuzumab and standard chemotherapy (cisplatin plus fluoropyrimidine) has recently completed the accrual[21]. Results of the trial are eagerly awaited. Novel anti-HER2 drugs have been developed to try to overcome secondary trastuzumab resistance, as in the case of trastuzumab-emtansine (T-DM1). Data from phase Ⅲ GATSBY trial were recently presented concluding that TDM-1 did not improve patients' outcome compared to second-line taxanes at the 2015 clinical cut-off[22].

The majority of gastric cancer patients who achieve an initial response to trastuzumab-based regimens develop resistance within 7 mo[23]. These unsatisfactory results may be attributed to primary (*de novo*) or secondary (acquired) resistance to the HER2-targeted therapy. Therefore, as it happened for breast cancer, the onset of trastuzumab resistance has been investigated also in gastric cancer, showing several molecular mechanisms underlying the acquired resistance to HER2 inhibitors[24]. Lee *et al*[25] identified that *HER2*-amplified GC patients have diverse pattern of various concurrent molecular events. Zuo *et al*[26] employed the human gastric carcinoma cell line NCI-N87 with high HER2 expression to create trastuzumab-resistant NCI-N87/TR cells by stepwise exposure to increasing doses of trastuzumab. They showed that activation of the PI3K-AKT signalling pathway downstream of HER2 was one of the major mechanisms leading to resistance of NCI-N87/TR gastric cancer cells to trastuzumab, which was probably associated with *PTEN* gene down-regulation and mutation, as well as with over-activity of the IGF-1R signalling pathway[26]. The study conducted by Piro *et al*[27] identified the FGFR3/AKT axis as an escape pathway responsible for trastuzumab resistance in gastric cancer, indicating that the inhibition of FGFR3 could be a potential strategy to modulate this resistance. Recently, Arienti *et al*[28] explored the role of the IQ-domain GTPase-activating protein 1 (IQGAP1), a multifunctional scaffold protein, which interacts with diverse proteins to regulate cell adhesion and cell migration. IQGAP1 governs HER-2 expression, phosphorylation and signalling in breast cancer cell lines[29], it is overexpressed in aggressive form

of gastric cancer[30] and its overexpression is correlated with trastuzumab-induced resistance in breast cancer cell lines[31]. The study of Arienti *et al*[28] revealed that high IQGAP1 expression leads to resistance to trastuzumab in gastric cancer; in addition, they found two new mutations of the *HER2* gene that may be correlated with acquired resistance to the drug. Moreover, a functional cross-talk between the receptor tyrosine kinase MET and HER family members has been reported in the context of the acquisition of aggressive phenotypes[32]. The hepatocyte growth factor (HGF) mediated activation of MET may also causeresistance to lapatinib in HER2-amplified GC cell lines by stimulating downstream signalling[33]. De Silva *et al*[34] confirmed *in vitro* that MET is likely to be a significant mechanism of lapatinib resistance *in vivo*. Finally, we recently showed that HER2 loss may be associated with acquired resistance to first-line trastuzumab-based treatment in patients with initially HER2-positive GC[35]. All these evidences enhance the complex cross-talk between HER2 and its downstream pathway and stress the importance of further elucidating the strategies to overcome resistance to HER2-targeted therapy. Indeed, identifying the mechanisms underlying treatment resistance would increase the benefit from HER2-targeted therapy in patients with HER2-positive gastric cancer. Certainly, development of inhibitors targeting multiple receptors or common downstream signalling proteins deserves further investigation.

EGFR

The EGFR (or ERBB1) belongs to RTKs and it is the second most frequent RTK playing a key role in GC initiation and progression. Despite the wide use of anti-EGFR monoclonal antibodies in colorectal cancer, demonstration of efficacy in GC has not yet been provided. EGFR overexpression has been reported in 24%-27% of all gastric adenocarcinomas[36]. Several studies have evaluated the efficacy and safety of different anti-EGFR therapy, based on preclinical data[37]. The phase Ⅲ EXPAND trial evaluated the addition of cetuximab to first-line capecitabine and cisplatin in a non-selected cohort of GC patients. This trial showed no significant advantage in median PFS (4.4 mo *vs* 5.6 mo in favor of control arm, P = 0.32)[38]. The REAL-3 phase Ⅲ trial evaluated the addition of panitumumab to epirubicin, oxaliplatin, and capecitabine (EOC). It demonstrated that the addition of panitumumab is detrimental as to OS (11.3 mo for EOC and 8.8 mo for EOC plus panitumumab, HR 1.37, 95%CI:1.07-1.76, P = 0.013)[39]. These disappointing results have been confirmed with another anti-EGFR drug, nimotuzumab[40]. The failure of anti-EGFR monoclonal antibodies in advanced GC may lie in the lack of a proper selection, as happened to the patients treated in the aforementioned trials. A recent publication from Birkman *et al*[41] studied the prevalence of *EGFR* overexpression/genomic amplification in gastric intestinal-type adenocarcinoma. In this work, 220 paraffin-embedded samples of GC were collected with the aim of elucidating the prevalence of *EGFR* overexpression/amplification, the *HER2* overexpression/amplification and the combination of the previous two. Interestingly, EGFR overexpression was more frequent in intestinal-type GCs (32.7% of the specimens) and its genomic amplification was demonstrated in 14.1% of the patients. It has also been shown that *EGFR* amplification was associated to a deeper tumor invasion (pT3-4 *vs* pT1-2, OR 2.15, P = 0.029). This unfavourable clinical feature correlated also to a shortened time to cancer recurrence (P = 0.026) and cancer specific survival (P = 0.033). Furthermore, *HER2* overexpression/amplification has been shown to be less frequent when compared to *EGFR* overexpression/amplification and *EGFR/HER2* co-amplification (3.6% of the cases), indicating that these two different populations may bear specific genomic alterations potentially approachable with different treatments. All these data strongly suggest that modern trials should be designed with a careful stratification according to *EGFR* amplification to properly assess the clinical effectiveness of anti-EGFR drugs in GC patients.

RAS and BRAF

KRAS mutation occurs in less than 5% of GC and may have a negative prognostic value in GC patients. KRAS activates critical pathways involved in carcinogenesis and tumor progression, such as PI3K-Akt, RAF, MEK-extracellular signal regulated kinase and NF-kB. However, no target therapies are currently approved for this molecular aberration[42]. Other drugs, such as MEK inhibitors were tested in *KRAS* mutated cancer cell lines with promising results. Since preclinical study suggested that the combination of MEK-inhibitors and PI3K or BCL-XL inhibitors may be efficacious in *KRAS* mutant lung cancer patients[43], it would be intriguing to evaluate MEK inhibitors in monotherapy or in combination with PI3K inhibitors or BCL-XL in GC patients who carry this mutation. In GC patients**,** *BRAF* mutations are rare (2.2% in TCGA database) and are mostly represented by *BRAF* V599M[42]. The role of this mutation in GC is yet to be assessed.

FGFR2

FGFR2 amplification is associated with tumor cell proliferation and survival of GC cell lines and indicates poor prognosis. In the TCGA classification, approximately 9% of CIN GC patients had *FGFR2* gene amplification. Several drugs and studies targeting this mutation are ongoing[5]. A phase Ⅱ randomized trial is evaluating the activity of AZD4547 (a FGFR 1-2 and 3 inhibitor) compared to paclitaxel in second-line treatment. Other ongoing trials are testing dovitinib in *FGFR2* amplified GC patients or in combination with docetaxel[18].

C-MET

Mesenchymal epithelial transition factor (MET) alteration was rarely observed in GC (8%)[44]. MET is an RTK that interacts with its native ligand HGF. Deregulated expression of C-MET in GC has been related to worse

prognosis. In fact, the HGF/c-MET signal is involved in cancer growth, invasion, angiogenesis, anti-apoptosis and epithelial to mesenchymal transition[45]. Two monoclonal antibodies, rilotumumab (an anti-HGF antibody) and onartuzumab (an anti c-MET antibody) were tested. In a phase Ⅰb/Ⅱ study, rilotumumab was effective and it improved PFS[46]. Based on these data, the phase Ⅲ RILOMET-1 trial, conducted on selected *c-MET* amplified patients, evaluated OS and ORR in the experimental arm with rilotumumab plus ECX compared to control arm with placebo plus ECX. The trial results were negative, and demonstrated that rilotumumab does not improve survival[47]. A similar phase Ⅲ study called RILOMET-2 is ongoing for Asian patients in the same setting[48].

Onartuzumab, a monoclonal antibody directed to c-MET, was tested in MET-Gastric study, in which patients were randomized to receive FOLFOX alone or in combination with onartuzumab. Once again, results were negative (OS: 11.0 mo in the experimental arm *vs* 11.3 mo in the control arm, HR = 0.82, *P* = 0.24)[49]. Recently results on a specific MET kinase inhibitor have been presented at ASCO 2016[50]. For the first time AMG337 was tested, in a phase Ⅰ study, in humans with solid tumors: 51 patients were treated and among them 10 had *MET*-amplified gastrointestinal cancers: 4 partial responses and 1 complete response were observed. At the end of the study a maximum tolerated dose of 300 mg was reached. Although an expansion phase on *MET*-amplified patients was on the way, it was early interrupted for excess of toxicity. Despite these negative results, the interest on c-MET as a potential molecular target for novel therapies has not vanished, since better molecular selection of the patients and optimal combination/drugs may finally achieve the expected results.

VEGF and VEGFR-2

Another frequently amplified gene in CIN subtype is *VEGF*, a mediator of angiogenesis that is essential for cancer growth and metastasis as it ensures oxygen and nutrients supply to proliferating cancer cells[51]. Bevacizumab, a monoclonal antibody that targets VEGF, was tested in the AVAGAST trial. This study did not meet its primary endpoint of improved OS (median OS 12.1 mo *vs* 10.1 mo, HR 0.87 95%CI: 0.73-1.03, *P* = 0.1), but improvements in median PFS and tumor response rate were reported[52]. Similarly, the AVATAR trial showed no survival benefit with antiangiogenic therapy added to cisplatin and capecitabine-based regimens (HR 1.1)[53]. Although the addition of bevacizumab to standard therapy showed disappointing results, antiangiogenic strategy was further investigated beyond first line treatment. Ramucirumab, a fully human monoclonal IgG directed against VEGFR-2, was evaluated both as single agent and in combination with chemotherapy[54-56]. In the REGARD trial, ramucirumab demonstrated a statistically significant improvement when compared to the best supportive care in pretreated GC patients with advanced disease (OS: 5.2 mo *vs* 3.8 mo respectively, HR = 0.776; *P* = 0.047)[54]. In the RAINBOW trial, patients were randomized to receive paclitaxel with or without ramucirumab. Median OS was 9.63 mo for the combination therapy and 7.36 mo for paclitaxel alone (HR = 0.807, 95%CI: 0.678-0.962; *P* = 0.017)[55]. Recently, a novel VEGFR-2 tyrosine kinase inhibitor, apatinib, was evaluated in Asian patients who had previously received 2 or 3 lines of chemotherapy[57]. Patients exposed to apatinib had an improved median OS (6.5 mo *vs* 4.7 mo; HR = 0.709; 95%CI: 0.537-0.937; *P* = 0.156) and median PFS (2.6 mo *vs* 1.8 mo; HR = 0.444; 95%CI: 0.331-0.595; *P* < 0.001) compared to patients who received placebo. Therefore, multitarget TKIs represent another potential approach to block angiogenesis by simultaneously targeting VEGFR and other signaling pathways. Notably, the role of antiangiogenic strategy seems to gain importance in subsequent lines of treatment, but its role in first-line therapy is still unclear. An ongoing randomized phase Ⅲ trial is assessing the potential survival benefit of ramucirumab in combination with cisplatin and capecitabine given upfront[56].

GC WITH MICROSATELLITE INSTABILITY

According to the TCGA's molecular classification, the enrichment for microsatellite instability (MSI) characterizes a distinct molecular subgroup of GC. MSI occurs in about 15%-30% of GCs, and more frequently correlates with intestinal histotype, location in the distal part of the stomach, female gender and older age at diagnosis[5,58,59].

MSI is a genetic alteration consisting of the expansion or contraction of regions of repetitive nucleotide sequences, called microsatellites. The alteration is triggered by a dysfunction of DNA mismatch repair (MMR) enzymes, caused by mutations in one of several different DNA mismatch repair genes (*i.e., MLH1* or *MSH2*). In a single cell, bi-allelic inactivation of *MMR* genes causes an increased mutation rate (genomic instability) due to the failure of DNA mismatch repair that usually occurs during normal DNA synthesis[60].

Defective DNA mismatch repair is the hallmark of Lynch syndrome. Moreover, approximately 15% of sporadic colorectal cancers also displays MSI since both alleles of a *MMR* gene are inactivated[61]. Different *MMR* genes are probably involved in MSI-high (MSI-H) sporadic gastric cancer without *MLH1* hypermethylation, which represents the main mechanism leading to MMR deficiency in MSI GC[62,63].

MSI-H colorectal cancer have better prognosis compared to MSI low, and should not receive adjuvant chemotherapy with fluoropyrimidine after resection for stage Ⅱ disease[64]. In gastric cancer, 5-FU is frequently used and information about sensitivity to this agent may be very useful. A meta-analysis of Zhu *et al*[65] showed a 37% mortality risk reduction and improved median OS in patients with MSI-H compared to MSI-L(low) or microsatellite stable (MSS) GC patients. The relationship between MMRd, MSI and survival has been examined in patients with resectable GC randomized to surgery alone or perioperative chemotherapy within the MRC MAGIC

trial. MSI and *MLH1* deficiency was associated with a better outcome in patients treated with surgery alone while it had a negative prognostic effect in those treated with chemotherapy[62].

Despite MSI cases generally lack of targetable amplifications, mutation in *PIK3CA*, *ERBB3, ERB22* and *EGFR* are noted[5,59]; *BRAF* V600E mutations, commonly seen in MSI colorectal cancer, are absent in MSI GC[5]. However, the predictive role of these mutations in MSI GC population is uncertain. The combination of olaparib with paclitaxel as second-line therapy was found to be more active compared with paclitaxel alone in patients with metastatic or recurrent GC. Although the trial did not meet its primary endpoint (namely PFS), olaparib prolonged survival in patients with low levels of ataxia telangiectasia mutated, a key activator of DNA damage response[66]. A phase Ⅲ trial in this setting is under way and detailed analysis in MSI GC could be attractive.

The hypothesis of an increased activity of immunotherapy in MSI non-colorectal cancer has recently generated interest. In fact, the increased number of somatic mutations may amplify the number of neoantigens, thus stimulating the immune system and conferring higher sensitivity to PD-1 blockade to tumor[67,68]. Interestingly, the tendency to have a lymphocytic infiltrate, observed in MSI tumors, likely reflects immune activation of T-cells directed against tumor-specific carboxy-terminal frameshift peptides that are associated with MSI[69]. In addition to that, genomic aberrations in tumor cells lead to aberrant PD-L1 expression, suggesting a predictive role for MSI.

MSI has already been reported as a strong predictive factor for the use of immune check-point inhibitors in the treatment of patients with colorectal cancer[70]. The immune-related objective response rate and immune-related 6-mo PFS rate were 40% and 78%, respectively, for patients with dMMR and 0% and 11% for those with MMR-proficient cancer, with a higher median PFS and survival in the cohort with dMMR colorectal cancers *vs* 2.2 and 5.0 mo, respectively, in the cohort with MMR-proficient tumors. Le *et al*[68] enrolled 41 consecutive patients (9 patients with MMR deficient solid tumors other than colorectal cancer, only 1 patient with GC) to explore the activity of PD-1 blockade according to MMR status in non colorectal cancer too. Although data are not ready for clinical application, 30% of GC have been shown to present with a burden of nonsynonymous mutations that may define who are the optimal candidates for immune checkpoint inhibitors treatment[71]. Of note, a phase 2 study of pembrolizumab in subjects with advanced gastric or gastroesophageal junction adenocarcinoma who progressed after first-line therapy with platinum and fluoropyrimidine is currently recruiting participants[72]. Muro *et al*[73] have recently reported the activity of pembrolizumab in GC in a phase Ⅰ trial. The authors showed a decrease in tumor burden in 41% of the study patients. The ORR was 32% in Asian patients and 30% in non-Asian patients[73]. A phase 2 trial of nivolumab or nivolumab plus ipilimumab is recruiting patients to evaluate the response to checkpoint inhibitors in MSI-H gastrointestinal cancers[74]. Interestingly, a preventive vaccine, set-up using neopeptides frequently affecting MSI tumorigenesis, has been shown to delay the onset of dMMR tumors. It remains to be proven if vaccination against these neopeptides might be a promising approach for novel adjuvant treatment strategies in patients with MSI-H tumors[75].

GC WITH GENOMIC STABILITY

GS GCs account for around 20% of all the tumors analyzed by the TCGA project. This subtype occurs with equal frequency in males and females. GS gastric tumors are enriched for the diffuse histological variant [58% according to Lauren's classification) and for the poor cohesive variant (58% according to World Health Organization (WHO) classification]. One quarter of GS GCs arise in the antrum, about 20% in the gastroesophageal junction/cardia, and approximately 15% in the gastric body/fundus. The principal somatic genomic alterations observed in GS gastric tumors involve *CDH1*, *ARID1A* and *RHOA*. In addition, a recurrent interchromosomal translocation (between *CLDN18* and *ARHGAP26*) implicated in cell motility was found in GS gastric tumors[5].

CDH1

The *CDH1* gene is located on chromosome 16q22.1 and encodes E-cadherin, which belongs to the cadherin superfamily of calcium-dependent cell adhesion molecules. E-cadherin plays a well-documented role in the progression of epithelial cancers. Inactivating mutations in the *CDH1* gene are frequently found in gastric cancer, especially in hereditary diffuse gastric cancer[76]. *CDH1* promoter methylation is also frequently found in sporadic gastric cancer[77]. During epithelial tumorigenesis, the protein is downregulated and E-cadherin has been categorized as a tumor suppressor gene[78]. Li *et al*[79] reported that in diffuse-type GC, *CDH1* mutation is associated with shortened patients survival, independently from disease stage. In the analysis of the TGCA Research Network *CDH1* somatic mutations were enriched in the GS subtype (37% of cases). Therefore, the prognostic value of *CDH1* as well as its potential as therapeutic target in gastric cancer has yet to be fully understood and explored.

ARID1A

Inactivating mutations of *ARID1A* were found in GS gastric cancer, as in the EBV-subtype[5]. The *ARID1A* gene, located in chromosome 1p35.3, encodes adenine-thymine-rich interactive domain-containing protein 1A, which participates in chromatin remodeling, therefore is involved in regulating cellular processes including DNA repair, differentiation, and development[80]. As shown by Wang *et al*[81], loss of *ARID1A* expression was significantly correlated with tumor stage and grade; moreover, it was also significantly correlated with poor survival in GC patients. Restoring *ARID1A* expression in gastric cancer cells significantly inhibited cell proliferation and colony formation, whereas silencing *ARID1A* expression

in gastric epithelial cell lines significantly enhanced cell growth rate[81].

RHOA

Rho belongs to the Ras-related family of small molecular weight GTP-binding proteins, and it works as a molecular switch between the GDP-bound inactive form and the GTP-bound active form[82]. It regulates cytoskeletal organization, cell adhesion, intracellular membrane trafficking, gene transcription, apoptosis, and cell cycle progression[83]; moreover, it activates STAT3 to promote tumorigenesis[84]. RhoA plays a role in these processes through a variety of effectors including ROCK1, mDia and protein kinase N[85]. mDia is involved in nucleation and polymerization of actin filaments, while ROCK intervenes in induction of actinomyosin bundles and contractility. The balance between mDia and ROCK regulates cell morphogenesis, adhesion, and motility activities. In addition, the Rho-ROCK pathway is involved in Ras-mediated transformation, the amoeboid movement of tumor cells in the three-dimensional matrix, and transmigration of tumor cells through the mesothelial monolayer[86]. According to the TCGA, RHOA mutations were clustered in two adjacent amino-terminal regions that are predicted to be at the interface of RHOA with ROCK1 and other effectors, leading to a modulation of signaling downstream of RHOA[5]. Interestingly, diffuse-type GCs, characterized by malignant phenotype and stromal differentiation, frequently have gain-of-function mutations of RHOA[87].

The TCGA network discovered a recurrent interchromosomal translocation between claudin 18 (*CLDN18*) and Rho GTPase-activating protein 6 (*ARHGAP26*), resulting in the *CLDN18-ARHGAP26* fusion gene, which primarily occurs in GS GC[5]. ARHGAP26 (also known as GTPase Regulator Associated with Focal Adhesion Kinase, GRAF) is a GTPase-activating protein that facilitates conversion of RHO GTPases to the GDP state and has been implicated in enhancing cellular motility[88]. CLDN18 is a component of the tight junction adhesion structures[89]. Yao *et al*[90] showed that expression of *CLDN18-ARHGAP26* fusion gene in gastric epithelial cells resulted in epithelial–mesenchymal transition, which is indicative of cell transformation in cancer development. A recent trial tested IMAB362, a chimeric IgG1 antibody against CLDN18.2 showing clinical activity in patients with 2 + /3 + immunostaining[91].

The *CLDN18–ARHGAP* fusions were mutually exclusive with *RHOA* mutations; within the GS subtype, 30% of cases had either *RHOA* or *CLDN18–ARHGAP* alterations[5].

Given the role of RHOA in cell motility, modulation of RHOA may contribute to the disparate growth patterns and lack of cellular cohesion that are hallmarks of diffuse tumors.

Rho/Rho-kinase inhibitors have been explored as putative therapeutic targets in various diseases, including cancers[92]. The development of drugs that inhibit Rho GTPase signaling would be of great potential in this setting.

Other notable patterns

The GS subtype exhibited elevated expression of cell adhesion pathways, including the B1/B3 integrins, syndecan-1-mediated signaling, and angiogenesis-related pathways. Also in the GS subtype, hierarchical clustering of samples and pathways revealed several notable patterns, including elevated expression of mitotic network components such as AURKA/B and E2F, targets of MYC activation, FOXM1 and PLK1 signaling and DNA damage response pathways[5]. Specific inhibitors of AURKA are currently under investigation in phase Ⅰ/Ⅱ clinical trials in advanced GC[93]. PLKs, mitotic kinases of the polo family, play a pivotal role in the normal cell cycle, and their overexpression is involved in the pathogenesis of multiple human cancers[94]. PLK1 is overexpressed in approximately 80% of human tumors, including gastric cancer, and it is associated with poor prognosis[94]. Currently, inhibitors of PLK1 are being developed[95]. In a phase Ⅰ trials enrolling patients with advanced solid cancers, including gastric cancer, volasertib, a potent and selective PLK inhibitor that induces mitotic arrest and apoptosis, demonstrated anti-cancer activity with a manageable safety profile[96].

EBV ASSOCIATED GC

Latent EBV infection is associated with about 10% of GCs, as demonstrated by *in situ* hybridization EBV encoded miRNA detection, by whole genome sequencing or by PCR EBV genome detection[5].

EBV associated GC has been related to different epidemiological and clinico-pathological features. In a meta-analysis of 39 case-control studies, Bae *et al*[97] investigated the strength of association between EBV infection and GC risk, and showed a 10 fold increase (95%CI: 5.89-17.29). It was also reported that there is a higher risk of EBV associated GC in Far East Asia if compared to Europe[98].

In a meta-analysis of 70 studies the pooled prevalence of EBV-positive GC resulted 8.7% (95%CI: 7.5%-10.0%) with similar distributions across the three analyzed geographic regions (America, Asia and Europe). Moreover, a two-fold difference in male/female ratio favored men as to prevalence of EBV positive GC. The antral location was less frequently associated with EBV infection when compared to other types. In contrast, there was no statistically significant difference in the proportion of EBV-positive disease between intestinal (9.5%; 95%CI: 7.2%-12.5%) and diffuse (7.6%; 95%CI: 5.7%-10.3%) histology[98].

In addition, EBV-positive GC was more prevalent in younger patients compared to older subjects[99].

As to possible therapeutic approaches, Kim *et al*[100] observed that EBV infected GC patients had a higher rate of alteration in pathways related to immune response which may also be related to a more favorable prognosis

in these patients. According to *TCGA*, *PD-L1* gene was frequently amplified in EBV-positive GC, adding proofs to the hypothesis of higher immunogenicity of this class of GC. Based on the evidence that 15% of EBV positive GC harbor amplification of chromosomal region 9p24.1, the locus of PD-L1 and PD-L2, potential role of PD-L1 expression in EBV-positive GC was investigated in a study[101]. In EBV-associated GC, PD-L1 expression was present in 50% (16/32) and 94% (30/32) of tumor and immune cells, respectively. In contrast, EBV-negative GC showed a lower PD-L1 expression (10% and 39% of tumor and immune cells, respectively, $P < 0.001$), thus providing a further rationale for testing PD-1 expression in this GC subtype to potentially identify a predictive response factor for immunomodulatory therapeutic strategies.

Besides PD-L1 and PD-L2 expression, *PIK3CA* mutations, DNA hypermethylation, and *JAK2* mutations are also present[5]. In a large retrospective study, 855 GC specimens were analyzed to verify protein expression levels and prognostic values of *PIK3CA*, JAK2, PD-L1 and PD-L2. Only 59 samples were found to be EBV positive. *PIK3CA* and PD-L2 were more highly expressed in EBV positive GC than in negative ones, but no prognostic value of *PIK3CA*, JAK2, PD-L1 or PD-L2 was found. No differences in JAK2, PD-L1 or PD-L2 expression were seen between EBV positive and negative cases. Moreover, the expression of *PIK3CA*, JAK2, PD-L1 or PD-L2 was not significantly associated with any clinico-pathological feature, maybe due to the small number of EBV-associated GC cases, and the prognostic value of these mutations remains uncertain[102].

THE ACRG CLASSIFICATION

The ACRG proposed a different molecular classification for gastric cancer in 2015[7]. This classification has some overlapping features with the one proposed by TCGA even though some differences can be highlighted. The clustering process included a first subdivision into MSI (22.7%, better prognosis, mainly intestinal type) and EMT tumours (15.3%, worse prognosis mainly diffused type) with two exclusive gene expression profiles, the first characterized by the loss of function of genes involved in the MMR and the second by alterations in cell adhesion, angiogenesis, and motility. Notably, the MSI subtype was associated with a hypermutation in genes such as: *KRAS* (23.3%), PI3K-PTEN-mTOR pathway (42%), *ALK* (16.3%) *ARID1A* (44.2%), *ERBB2* (16.3%) and *ERBB3* (14%). The remaining tumours were further divided into MSS/TP53$^+$ (26.3%, P53 function intact) and MSS/TP53$^-$ (35.7%, loss of oncosuppressor function). In terms of survival, the MSI subtype showed the best overall prognosis, followed by MSS/TP53$^+$, MSS/TP53$^-$ and MSS/EMT. The MSI/TP53$^+$ subtype was more frequently associated with EBV infection if compared to the other groups and showed an active *TP53* pathway and a higher prevalence (compared to MSI/TP53$^-$) of *APC*, *ARID1A*, *KRAS*, *PI3KCA*, and *SMAD4* mutations. Finally, the MSI/TP53$^-$ subtype showed the highest prevalence of *TP53* mutations, relevant copy number variations (CNVs), a greater aneuploidy and recurrent focal amplifications in *MDM2, ROBO2, GATA6, MYC. ERBB2, EGFR, CCNE1* and *CCND1*. These latter two amplifications were mutually exclusive, so they could be considered driver alterations.

A comparison of the ACRG categories with the TCGA subtypes showed similarities in the tumors with MSI, while GS was approximated to MSS/EMT, EBV to MSS/TP53$^+$, and CIN to MSS/TP53$^-$. Nevertheless, in the TCGA cohort the EBV positive cancers represented a separated subgroup (with a favourable phenotype), whereas in the ACRG classification EBV infection occurred more frequently in the MSS/TP53$^+$ subtype, without CNVs, hypermethylation or hypermutation. Moreover, *PI3KCA* and *ARID1A* mutations were more prevalent in EBV$^+$ gastric cancers compared to MSS subtypes.

Although both the MSS/EMT and the GS molecular subgroups included tumors with a prevalent diffuse histology, the TCGA classification showed a lower percentage of Lauren's diffuse subtype compared to the ACRG database (24% *vs* 45% respectively); additionally, *CDH1* and *RHOA* mutations did not appear prevalent in the MSS/EMT subgroup, unlike the GS subtype. Finally, GS tumours were also present in the ACRG MSS/EMT, MSS/Tp53 + and MSS/tp53$^-$ molecular subgroups. All these findings showed that the GS and the MSS/EMT subgroups were not equivalent.

The comparison of the CIN TCGA subtype to ACRG MSS/TP53$^-$ subtype showed that the first is quite homogeneously distributed in the subtypes classified by ACRG.

Overall survival associations were weaker when using the TCGA genomic scheme in the ACRG cohort compared to the original prognosis trends: While the MSI subtype showed a better prognosis in both classifications, there were no differences in prognosis in CIN and GS subtypes when they were identified based on application of the TCGA classification on the ACRG patient population.

CONCLUSION

While the advent of novel molecular classifications has faded the "one size fit-all" era, a more profound understanding of the underpinning tumour biology has set the dawn of a more contemporary clinical approach called precision medicine. At present, the two aforementioned genomic classifications of GC represent the state-of-the-art achieved so far. Somehow it is possible to find an overlap between the TCGA and ACRG subtypes even though some difference can still be found. Emerging data clearly individuate a category of GC characterized by MSI that may benefit from immunotherapeutic approaches. For this subgroup, with good prognosis, the development of anti PD-1/PD-L1 drugs could be the leading research avenue. High mutational burden is also a driving feature of EBV positive GC that could be targeted with immunotherapy as

Table 1 Clinical outcomes of recent trials in gastric and esophagogastric adenocarcinomas

Trial name	Phase of study	Line of treatment	Selected biomarker	Treatment arms	*n*	Primary endpoint	Outcomes
CIN							
TOGA[3]	Ⅲ	First	HER2 expression/ amplification	CF/CX CF/CX + trastuzumab	296 298	OS	OS: 13.8 mo *vs* 11.1 mo (HR = 0.74, *P* = 0.005) PFS: 6.7 mo *vs* 5.5 mo (HR = 0.71, *P* = 0.0002) ORR: 47% *vs* 35% (*P* = 0.001)
LOGiC[19]	Ⅲ	First	HER2 expression/ amplification	CapeOX CapeOx + lapatinib	273 272	OS	OS: 12.2 mo *vs* 10.5 mo (HR = 0.91, *P* = 0.34) PFS: 6.0 mo *vs* 5.4 mo (HR = 0.82, *P* = 0.038) ORR: 53% *vs* 39% (*P* = 0.003)
TyTAN[20]	Ⅲ	Second	HER2 amplification by FISH	Paclitaxel Paclitaxel + lapatinib	129 132	OS	OS: 11.0 mo *vs* 8.9 mo (HR = 0.84, *P* = 0.104) PFS: 5.4 mo *vs* 4.4 mo (HR = 0.85, *P* = 0.244) ORR: 27% *vs* 9% (*P* < 0.001)
JACOB[21]	Ⅲ	First	HER2 expression/ amplification	Pertuzumab + tFP Placebo + tFP		OS	Ongoing
GATSBY[22]	Ⅱ / Ⅲ	Second	HER2 expression/ amplification	TAX T-DM1	117 228	OS	OS: 8.6 mo *vs* 7.9 mo (HR = 1.15, *P* = 0.86) PFS: 2.9 mo *vs* 2.7 mo (HR = 1.13, *P* = 0.31) ORR: 19.6% *vs* 20.6%
EXPAND[38]	Ⅲ	First	Unselected	CX CX + cetuximab	449 445	PFS	OS: 10.7 mo *vs* 9.4 mo (HR = 1.0, *P* = 0.95) PFS: 5.6 mo *vs* 4.4 mo (HR = 1.09, *P* = 0.32)
REAL-3[39]	Ⅲ	First	Unselected	EOC EOC + panitumumab	275 278	OS	OS: 11.3 mo *vs* 8.8 mo (HR = 1.37, *P* = 0.013) PFS: 7.4 mo *vs* 6.0 mo (HR = 1.22, *P* = 0.068) ORR: 42% *vs* 46% (*P* = 0.42)
RILOMET -1[47]	Ⅲ	First	MET positive by IHC HER2 negative	ECX ECX + rilotumumab	305 304	OS	OS: 11.5 mo *vs* 9.6 mo (HR = 1.37, *P* = 0.016) PFS: 5.7 mo *vs* 5.7 mo (HR = 1.30, *P* = 0.016) ORR: 39.2% *vs* 30% (OR = 0.67, *P* = 0.027)
METGastric[49]	Ⅲ	First	MET positive by IHC HER2 negative	mFOLFOX mFOLFOX + ornatuzumab	562	OS	OS: 11.3 mo *vs* 11.0 mo (HR = 0.82, *P* = 0.244) PFS: 6.8 mo *vs* 6.7 mo (HR = 0.90, *P* = 0.429) ORR: 41% *vs* 46% (*P* = 0.253)
AVAGAST[52]	Ⅲ	First	Unselected	CX CX + bevacizumab	387 387	OS	OS: 10.1 mo *vs* 12.1 mo (HR = 0.87, *P* = 0.1) PFS: 5.3 mo *vs* 6.7 mo (HR = 0.80, *P* = 0.037) ORR: 37.4% *vs* 46.0% (*P* = 0.03)
AVATAR[53]	Ⅲ	First	Unselected	CX CX + bevacizumab	102 100	OS	OS: 11.4 mo *vs* 10.5 mo (HR = 1.11, *P* = 0.55) PFS: 6.0 mo *vs* 6.3 mo (HR = 0.89, *P* = 0.47) ORR: 34% *vs* 41% (*P* = 0.35)
REGARD[54]	Ⅲ	Progression after TP	Unselected	BSC BSC + ramucirumab	117 238	OS	OS: 3.8 mo *vs* 5.2 mo (HR = 0.77, *P* = 0.047) PFS: 1.3 mo *vs* 2.1 mo (HR = 0.48, *P* < 0.001)
RAINBOW[55]	Ⅲ	Second	Unselected	Paclitaxel Paclitaxel + ramucirumab	335 330	OS	OS: 7.4 mo *vs* 9.6 mo (HR = 0.80, *P* = 0.017) PFS: 2.9 mo *vs* 4.4 mo (HR = 0.63, *P* < 0.0001)
Apatinib[57]	Ⅲ	Third or more	Unselected	Placebo Apatinib	91 176	OS	OS: 4.7 mo *vs* 6.5 mo (HR = 0.70, *P* = 0.015) PFS: 1.8 mo *vs* 2.6 mo (HR = 0.44, *P* < 0.001) ORR: 0% *vs* 2.84% (*P* = 0.16)
MSI							
NCT01063517[66]	Ⅱ	Second	ATM expression	Paclitaxel Paclitaxel + olaparib	62 61	PFS	OS: 8.3 mo *vs* 13.1 mo (HR = 0.56, *P* = 0.01) PFS: 3.55 mo *vs* 3.91 mo (HR = 0.80, *P* = 0.13)
NCT02589496	Ⅱ	Second	Unselected	Pembrolizumab		RR	Ongoing
GS							
FAST[91]	Ⅱ	First	CLDN18.2	EOX EOX + IMAB362	161	PFS	OS: 8.7 mo *vs* 12.5 mo (HR = 0.5) PFS: 5.7 mo *vs* 7.9 mo (HR = 0.5, *P* = 0.001)

Most significant target-oriented phase Ⅱ and phase Ⅲ trials are presented. In the table are shown in order: name of the trial, phase of the study, line of treatment, biomarker selection, treatment arms, number of enrolled patients, primary endpoint and key outcome results. tPF: Trastuzumab + Platinum + fluorouraci; PF: Platinum + fluoropyrimidine; TAX: Taxane, CF: Cisplatin + fluorouracil; CX: Cisplatin + capecitabine; EOC (or ECX): Epirubicin + oxaliplatin + capecitabine; BSC: Best supportive care; CIN: Chromosomal instability; GS: Genomical stability; MSI: Microsatellite instability.

efficaciously as in MSI tumours.

It is also possible to clearly segregate another class of GC classified either as GS or MMS/EMT, in which the prevalent deregulation is represented by EMT pathway alterations. Development of inhibitors of HGF/c-Met pathway, Rho/Rho-kinase, AURKA/AURKB, PLK1 could be a strategy adopted in the near future.

The category corresponding to CIN, and partially to MSS/TP53$^-$, represents a cluster of GC with high CNV variation leading to deregulation of specific biological targets such as receptors and kinases. Since these driver alterations are mostly mutually exclusive, they could be easily targeted using specific monoclonal antibodies or TKIs. On the other side, tumour heterogeneity may limit the efficacy of targeted strategies through alternative mechanisms of primary and acquired resistance[103].

The overall landscape is complex and our knowledge on this topic is still just at the starting point and novel trials should be designed accordingly (Table 1)[3,19-22,38,39,47,49,52-55,57,66,91]. Doubtlessly, dissecting and genotyping different

tumour subtypes and setting apart patients with different diseases will represent the future of gastrointestinal oncology. The key landmark comprehensive efforts made by TCGA and ACRG have just paved the way for precision oncology.

REFERENCES

1 **Siegel RL**, Miller KD, Jemal A. Cancer statistics, 2016. *CA Cancer J Clin* 2016; **66**: 7-30 [PMID: 26742998 DOI: 10.3322/caac.21332]

2 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]

3 **Bang YJ**, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, Lordick F, Ohtsu A, Omuro Y, Satoh T, Aprile G, Kulikov E, Hill J, Lehle M, Rüschoff J, Kang YK. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. *Lancet* 2010; **376**: 687-697 [PMID: 20728210 DOI: 10.1016/S0140-6736(10)61121-X]

4 **Sorlie T**, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lønning PE, Brown PO, Børresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA* 2003; **100**: 8418-8423 [PMID: 12829800 DOI: 10.1073/pnas.0932692100]

5 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]

6 **Lei Z**, Tan IB, Das K, Deng N, Zouridis H, Pattison S, Chua C, Feng Z, Guan YK, Ooi CH, Ivanova T, Zhang S, Lee M, Wu J, Ngo A, Manesh S, Tan E, Teh BT, So JB, Goh LK, Boussioutas A, Lim TK, Flotow H, Tan P, Rozen SG. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil. *Gastroenterology* 2013; **145**: 554-565 [PMID: 23684942 DOI: 10.1053/j.gastro.2013.05.010]

7 **Cristescu R**, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 2015; **21**: 449-456 [PMID: 25894828 DOI: 10.1038/nm.3850]

8 **Dunne PD**, McArt DG, Bradley CA, O'Reilly PG, Barrett HL, Cummins R, O'Grady T, Arthur K, Loughrey MB, Allen WL, McDade SS, Waugh DJ, Hamilton PW, Longley DB, Kay EW, Johnston PG, Lawler M, Salto-Tellez M, Van Schaeybroeck S. Challenging the Cancer Molecular Stratification Dogma: Intratumoral Heterogeneity Undermines Consensus Molecular Subtypes and Potential Diagnostic Value in Colorectal Cancer. *Clin Cancer Res* 2016; **22**: 4095-4104 [PMID: 27151745 DOI: 10.1158/1078-0432.CCR-16-0032]

9 **Uhlik MT**, Liu J, Falcon BL, Iyer S, Stewart J, Celikkaya H, O'Mahony M, Sevinsky C, Lowes C, Douglass L, Jeffries C, Bodenmiller D, Chintharlapalli S, Fischl A, Gerald D, Xue Q, Lee JY, Santamaria-Pang A, Al-Kofahi Y, Sui Y, Desai K, Doman T, Aggarwal A, Carter JH, Pytowski B, Jaminet SC, Ginty F, Nasir A, Nagy JA, Dvorak HF, Benjamin LE. Stromal-Based Signatures for the Classification of Gastric Cancer. *Cancer Res* 2016; **76**: 2573-2586 [PMID: 27197264 DOI: 10.1158/0008-5472.CAN-16-0022]

10 **Lim B**, Kim JH, Kim M, Kim SY. Genomic and epigenomic heterogeneity in molecular subtypes of gastric cancer. *World J Gastroenterol* 2016; **22**: 1190-1201 [PMID: 26811657 DOI: 10.3748/wjg.v22.i3.1190]

11 **Chia NY**, Tan P. Molecular classification of gastric cancer. *Ann Oncol* 2016; **27**: 763-769 [PMID: 26861606 DOI: 10.1093/annonc/mdw040]

12 **Giam M**, Rancati G. Aneuploidy and chromosomal instability in cancer: a jackpot to chaos. *Cell Div* 2015; **10**: 3 [PMID: 26015801 DOI: 10.1186/s13008-015-0009-7]

13 **Aprile G**, Giampieri R, Bonotto M, Bittoni A, Ongaro E, Cardellino GG, Graziano F, Giuliani F, Fasola G, Cascinu S, Scartozzi M. The challenge of targeted therapies for gastric cancer patients: the beginning of a long journey. *Expert Opin Investig Drugs* 2014; **23**: 925-942 [PMID: 24806575 DOI: 10.1517/13543784.2014.912631]

14 **Chen T**, Xu XY, Zhou PH. Emerging molecular classifications and therapeutic implications for gastric cancer. *Chin J Cancer* 2016; **35**: 49 [PMID: 27233623 DOI: 10.1186/s40880-016-0111-5]

15 **Tan P**, Yeoh KG. Genetics and Molecular Pathogenesis of Gastric Adenocarcinoma. *Gastroenterology* 2015; **149**: 1153-1162.e3 [PMID: 26073375 DOI: 10.1053/j.gastro.2015.05.059]

16 **Hayakawa Y**, Sethi N, Sepulveda AR, Bass AJ, Wang TC. Oesophageal adenocarcinoma and gastric cancer: should we mind the gap? *Nat Rev Cancer* 2016; **16**: 305-318 [PMID: 27112208 DOI: 10.1038/nrc.2016.24]

17 **Gravalos C**, Jimeno A. HER2 in gastric cancer: a new prognostic factor and a novel therapeutic target. *Ann Oncol* 2008; **19**: 1523-1529 [PMID: 18441328 DOI: 10.1093/annonc/mdn169]

18 **Fontana E**, Smyth EC. Novel targets in the treatment of advanced gastric cancer: a perspective review. *Ther Adv Med Oncol* 2016; **8**: 113-125 [PMID: 26929787 DOI: 10.1177/1758834015616935]

19 **Hecht JR**, Bang YJ, Qin SK, Chung HC, Xu JM, Park JO, Jeziorski K, Shparyk Y, Hoff PM, Sobrero A, Salman P, Li J, Protsenko SA, Wainberg ZA, Buyse M, Afenjar K, Houé V, Garcia A, Kaneko T, Huang Y, Khan-Wasti S, Santillana S, Press MF, Slamon D. Lapatinib in Combination With Capecitabine Plus Oxaliplatin in Human Epidermal Growth Factor Receptor 2-Positive Advanced or Metastatic Gastric, Esophageal, or Gastroesophageal Adenocarcinoma: TRIO-013/LOGiC--A Randomized Phase III Trial. *J Clin Oncol* 2016; **34**: 443-451 [PMID: 26628478 DOI: 10.1200/JCO.2015.62.6598]

20 **Satoh T**, Xu RH, Chung HC, Sun GP, Doi T, Xu JM, Tsuji A, Omuro Y, Li J, Wang JW, Miwa H, Qin SK, Chung IJ, Yeh KH, Feng JF, Mukaiyama A, Kobayashi M, Ohtsu A, Bang YJ. Lapatinib plus paclitaxel versus paclitaxel alone in the second-line treatment of HER2-amplified advanced gastric cancer in Asian populations: TyTAN--a randomized, phase III study. *J Clin Oncol* 2014; **32**: 2039-2049 [PMID: 24868024 DOI: 10.1200/JCO.2013.53.6136]

21 **Hoffmann-La Roche**. A Study of Perjeta (Pertuzumab) in Combination With Herceptin (Trastuzumab) and Chemotherapy in Patients With HER2-Positive Metastatic Gastroesophageal Junction or Gastric Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jul 31]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01774786 NLM Identifier: NCT01774786

22 **Hoffmann-La Roche**. A Study of Trastuzumab Emtansine *vs* Taxane in Patients With Advanced Gastric Cancer - Full Text View - ClinicalTrials.gov [Internet]. [accessed 2016 Jul 31]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT01641939

23 **Shimoyama S**. Unraveling trastuzumab and lapatinib inefficiency in gastric cancer: Future steps (Review). *Mol Clin Oncol* 2014; **2**: 175-181 [PMID: 24649329 DOI: 10.3892/mco.2013.218]

24 **Fanotto V**, Ongaro E, Rihawi K, Avallone A, Silvestris N, Fornaro L, Vasile E, Antonuzzo L, Leone F, Rosati G, Giuliani F, Bordonaro R, Scartozzi M, De Maglio G, Negri FV, Fasola G, Aprile G. HER-2 inhibition in gastric and colorectal cancers: tangible achievements, novel acquisitions and future perspectives. *Oncotarget* 2016; **7**: 69060-69074 [PMID: 27542243 DOI: 10.18632/oncotarget.11264]

25 **Lee JY**, Hong M, Kim ST, Park SH, Kang WK, Kim KM, Lee J. The impact of concomitant genomic alterations on treatment outcome for trastuzumab therapy in HER2-positive gastric cancer. *Sci Rep* 2015; **5**: 9289 [PMID: 25786580 DOI: 10.1038/srep09289]

26 **Zuo Q**, Liu J, Zhang J, Wu M, Guo L, Liao W. Development

of trastuzumab-resistant human gastric carcinoma cell lines and mechanisms of drug resistance. *Sci Rep* 2015; **5**: 11634 [PMID: 26108989 DOI: 10.1038/srep11634]

27 **Piro G**, Carbone C, Cataldo I, Di Nicolantonio F, Giacopuzzi S, Aprile G, Simionato F, Boschi F, Zanotto M, Mina MM, Santoro R, Merz V, Sbarbati A, de Manzoni G, Scarpa A, Tortora G, Melisi D. An FGFR3 Autocrine Loop Sustains Acquired Resistance to Trastuzumab in Gastric Cancer Patients. *Clin Cancer Res* 2016; **22**: 6164-6175 [PMID: 27267856 DOI: 10.1158/1078-0432.CCR-16-0178]

28 **Arienti C**, Zanoni M, Pignatta S, Del Rio A, Carloni S, Tebaldi M, Tedaldi G, Tesei A. Preclinical evidence of multiple mechanisms underlying trastuzumab resistance in gastric cancer. *Oncotarget* 2016; **7**: 18424-18439 [PMID: 26919099 DOI: 10.18632/oncotarget.7575]

29 **White CD**, Brown MD, Sacks DB. IQGAPs in cancer: a family of scaffold proteins underlying tumorigenesis. *FEBS Lett* 2009; **583**: 1817-1824 [PMID: 19433088 DOI: 10.1016/j.febslet.2009.05.007]

30 **Walch A**, Seidl S, Hermannstädter C, Rauser S, Deplazes J, Langer R, von Weyhern CH, Sarbia M, Busch R, Feith M, Gillen S, Höfler H, Luber B. Combined analysis of Rac1, IQGAP1, Tiam1 and E-cadherin expression in gastric cancer. *Mod Pathol* 2008; **21**: 544-552 [PMID: 18246045 DOI: 10.1038/modpathol.2008.3]

31 **White CD**, Li Z, Dillon DA, Sacks DB. IQGAP1 protein binds human epidermal growth factor receptor 2 (HER2) and modulates trastuzumab resistance. *J Biol Chem* 2011; **286**: 29734-29747 [PMID: 21724847 DOI: 10.1074/jbc.M111.220939]

32 **Khoury H**, Naujokas MA, Zuo D, Sangwan V, Frigault MM, Petkiewicz S, Dankort DL, Muller WJ, Park M. HGF converts ErbB2/Neu epithelial morphogenesis to cell invasion. *Mol Biol Cell* 2005; **16**: 550-561 [PMID: 15548598 DOI: 10.1091/mbc.E04-07-0567]

33 **Chen CT**, Kim H, Liska D, Gao S, Christensen JG, Weiser MR. MET activation mediates resistance to lapatinib inhibition of HER2-amplified gastric cancer cells. *Mol Cancer Ther* 2012; **11**: 660-669 [PMID: 22238368 DOI: 10.1158/1535-7163.MCT-11-0754]

34 **De Silva N**, Schulz L, Paterson A, Qain W, Secrier M, Godfrey E, Cheow H, O'Donovan M, Lao-Sirieix P, Jobanputra M, Hochhauser D, Fitzgerald R, Ford H. Molecular effects of Lapatinib in the treatment of HER2 overexpressing oesophago-gastric adenocarcinoma. *Br J Cancer* 2015; **113**: 1305-1312 [PMID: 26484410 DOI: 10.1038/bjc.2015.342]

35 **Pietrantonio F**, Caporale M, Morano F, Scartozzi M, Gloghini A, De Vita F, Giommoni E, Fornaro L, Aprile G, Melisi D, Berenato R, Mennitto A, Volpi CC, Laterza MM, Pusceddu V, Antonuzzo L, Vasile E, Ongaro E, Simionato F, de Braud F, Torri V, Di Bartolomeo M. HER2 loss in HER2-positive gastric or gastroesophageal cancer after trastuzumab therapy: Implication for further clinical research. *Int J Cancer* 2016; **139**: 2859-2864 [PMID: 27578417 DOI: 10.1002/ijc.30408]

36 **Kim MA**, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WH. EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. *Histopathology* 2008; **52**: 738-746 [PMID: 18397279 DOI: 10.1111/j.1365-2559.2008.03021.x]

37 **Zhang L**, Yang J, Cai J, Song X, Deng J, Huang X, Chen D, Yang M, Wery JP, Li S, Wu A, Li Z, Li Z, Liu Y, Chen Y, Li Q, Ji J. A subset of gastric cancers with EGFR amplification and overexpression respond to cetuximab therapy. *Sci Rep* 2013; **3**: 2992 [PMID: 24141978 DOI: 10.1038/srep02992]

38 **Lordick F**, Kang YK, Chung HC, Salman P, Oh SC, Bodoky G, Kurteva G, Volovat C, Moiseyenko VM, Gorbunova V, Park JO, Sawaki A, Celik I, Götte H, Melezínková H, Moehler M. Capecitabine and cisplatin with or without cetuximab for patients with previously untreated advanced gastric cancer (EXPAND): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 490-499 [PMID: 23594786 DOI: 10.1016/S1470-2045(13)70102-5]

39 **Waddell T**, Chau I, Cunningham D, Gonzalez D, Okines AF, Okines C, Wotherspoon A, Saffery C, Middleton G, Wadsley J, Ferry D, Mansoor W, Crosby T, Coxon F, Smith D, Waters J, Iveson T, Falk S, Slater S, Peckitt C, Barbachano Y. Epirubicin, oxaliplatin, and capecitabine with or without panitumumab for patients with previously untreated advanced oesophagogastric cancer (REAL3): a randomised, open-label phase 3 trial. *Lancet Oncol* 2013; **14**: 481-489 [PMID: 23594787 DOI: 10.1016/S1470-2045(13)70096-2]

40 **Satoh T**, Lee KH, Rha SY, Sasaki Y, Park SH, Komatsu Y, Yasui H, Kim TY, Yamaguchi K, Fuse N, Yamada Y, Ura T, Kim SY, Munakata M, Saitoh S, Nishio K, Morita S, Yamamoto E, Zhang Q, Kim JM, Kim YH, Sakata Y. Randomized phase II trial of nimotuzumab plus irinotecan versus irinotecan alone as second-line therapy for patients with advanced gastric cancer. *Gastric Cancer* 2015; **18**: 824-832 [PMID: 25185971 DOI: 10.1007/s10120-014-0420-9]

41 **Birkman EM**, Ålgars A, Lintunen M, Ristamäki R, Sundström J, Carpén O. EGFR gene amplification is relatively common and associates with outcome in intestinal adenocarcinoma of the stomach, gastro-oesophageal junction and distal oesophagus. *BMC Cancer* 2016; **16**: 406 [PMID: 27387915 DOI: 10.1186/s12885-016-2456-1]

42 **Choi YY**, Noh SH, Cheong JH. Molecular Dimensions of Gastric Cancer: Translational and Clinical Perspectives. *J Pathol Transl Med* 2016; **50**: 1-9 [PMID: 26498010 DOI: 10.4132/jptm.2015.09.10]

43 **Jiang ZB**, Huang J, Xie C, Li X, Liu L, He J, Pan H, Huang L, Fan XX, Yao XJ, Xie Y, Li N, Liu L, He JX, Leung EL. Combined use of PI3K and MEK inhibitors synergistically inhibits lung cancer with EGFR and KRAS mutations. *Oncol Rep* 2016; **36**: 365-375 [PMID: 27121230 DOI: 10.3892/or.2016.4770]

44 **Peng Z**, Zhu Y, Wang Q, Gao J, Li Y, Li Y, Ge S, Shen L. Prognostic significance of MET amplification and expression in gastric cancer: a systematic review with meta-analysis. *PLoS One* 2014; **9**: e84502 [PMID: 24416238 DOI: 10.1371/journal.pone.0084502]

45 **Joo MK**, Park JJ, Chun HJ. Recent updates of precision therapy for gastric cancer: Towards optimal tailored management. *World J Gastroenterol* 2016; **22**: 4638-4650 [PMID: 27217696 DOI: 10.3748/wjg.v22.i19.4638]

46 **Iveson T**, Donehower RC, Davidenko I, Tjulandin S, Deptala A, Harrison M, Nirni S, Lakshmaiah K, Thomas A, Jiang Y, Zhu M, Tang R, Anderson A, Dubey S, Oliner KS, Loh E. Rilotumumab in combination with epirubicin, cisplatin, and capecitabine as first-line treatment for gastric or oesophagogastric junction adenocarcinoma: an open-label, dose de-escalation phase 1b study and a double-blind, randomised phase 2 study. *Lancet Oncol* 2014; **15**: 1007-1018 [PMID: 24965569 DOI: 10.1016/S1470-2045(14)70023-3]

47 Phase III, randomized, double-blind, multicenter, placebo (P)-controlled trial of rilotumumab (R) plus epirubicin, cisplatin and capecitabine (ECX) as first-line therapy in patients (pts) with advanced MET-positive (pos) gastric or gastroesophageal junction (G/GEJ) cancer: RILOMET-1 study. *J Clin Oncol* [Internet]. [accessed 2016 Jul 31]. Available from: URL: http://meetinglibrary.asco.org/content/147255-156

48 **Amgen**. A Phase 3 Study of Rilotumumab (AMG 102) With Cisplatin and Capecitabine (CX) as First-line Therapy in Gastric Cancer. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jul 31]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT02137343 NLM Identifier: NCT02137343

49 METGastric: A phase III study of onartuzumab plus mFOLFOX6 in patients with metastatic HER2-negative (HER2-) and MET-positive (MET) adenocarcinoma of the stomach or gastroesophageal junction (GEC). *J Clin Oncol* [Internet]. [accessed 2016 Jul 31]. Available from: URL: http://meetinglibrary.asco.org/content/147779-156

50 Clinical activity of AMG 337, an oral MET kinase inhibitor, in adult patients (pts) with MET-amplified gastroesophageal junction (GEJ), gastric (G), or esophageal (E) cancer. *J Clin Oncol* [Internet]. [accessed 2016 Aug 15]. Available from: URL: http://meetinglibrary.asco.org/content/138984-158

51 **Aprile G**, Ongaro E, Del Re M, Lutrino SE, Bonotto M, Ferrari L, Rihawi K, Cardellino GG, Pella N, Danesi R, Fasola G. Angiogenic

inhibitors in gastric cancers and gastroesophageal junction carcinomas: A critical insight. *Crit Rev Oncol Hematol* 2015; **95**: 165-178 [PMID: 25800976 DOI: 10.1016/j.critrevonc.2015.02.009]

52 **Ohtsu A**, Shah MA, Van Cutsem E, Rha SY, Sawaki A, Park SR, Lim HY, Yamada Y, Wu J, Langer B, Starnawski M, Kang YK. Bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study. *J Clin Oncol* 2011; **29**: 3968-3976 [PMID: 21844504 DOI: 10.1200/JCO.2011.36.2236]

53 **Shen L**, Li J, Xu J, Pan H, Dai G, Qin S, Wang L, Wang J, Yang Z, Shu Y, Xu R, Chen L, Liu Y, Yu S, Bu L, Piao Y. Bevacizumab plus capecitabine and cisplatin in Chinese patients with inoperable locally advanced or metastatic gastric or gastroesophageal junction cancer: randomized, double-blind, phase III study (AVATAR study). *Gastric Cancer* 2015; **18**: 168-176 [PMID: 24557418 DOI: 10.1007/s10120-014-0351-5]

54 **Fuchs CS**, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, Safran H, dos Santos LV, Aprile G, Ferry DR, Melichar B, Tehfe M, Topuzov E, Zalcberg JR, Chau I, Campbell W, Sivanandan C, Pikiel J, Koshiji M, Hsu Y, Liepa AM, Gao L, Schwartz JD, Tabernero J. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial. *Lancet* 2014; **383**: 31-39 [PMID: 24094768 DOI: 10.1016/S0140-6736(13)61719-5]

55 **Wilke H**, Muro K, Van Cutsem E, Oh SC, Bodoky G, Shimada Y, Hironaka S, Sugimoto N, Lipatov O, Kim TY, Cunningham D, Rougier P, Komatsu Y, Ajani J, Emig M, Carlesi R, Ferry D, Chandrawansa K, Schwartz JD, Ohtsu A. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW): a double-blind, randomised phase 3 trial. *Lancet Oncol* 2014; **15**: 1224-1235 [PMID: 25240821 DOI: 10.1016/S1470-2045(14)70420-6]

56 **Aprile G**, Ferrari L, Cremolini C, Bergamo F, Fontanella C, Battaglin F, Rihawi K, Lonardi S, Loupakis F, Scartozzi M. Ramucirumab for the treatment of gastric cancers, colorectal adenocarcinomas, and other gastrointestinal malignancies. *Expert Rev Clin Pharmacol* 2016; **9**: 877-885 [PMID: 27149032 DOI: 10.1080/17512433.2016.1182861]

57 **Li J**, Qin S, Xu J, Xiong J, Wu C, Bai Y, Liu W, Tong J, Liu Y, Xu R, Wang Z, Wang Q, Ouyang X, Yang Y, Ba Y, Liang J, Lin X, Luo D, Zheng R, Wang X, Sun G, Wang L, Zheng L, Guo H, Wu J, Xu N, Yang J, Zhang H, Cheng Y, Wang N, Chen L, Fan Z, Sun P, Yu H. Randomized, Double-Blind, Placebo-Controlled Phase III Trial of Apatinib in Patients With Chemotherapy-Refractory Advanced or Metastatic Adenocarcinoma of the Stomach or Gastroesophageal Junction. *J Clin Oncol* 2016; **34**: 1448-1454 [PMID: 26884585 DOI: 10.1200/JCO.2015.63.5995]

58 **Pedrazzani C**, Corso G, Velho S, Leite M, Pascale V, Bettarini F, Marrelli D, Seruca R, Roviello F. Evidence of tumor microsatellite instability in gastric cancer with familial aggregation. *Fam Cancer* 2009; **8**: 215-220 [PMID: 19152022 DOI: 10.1007/s10689-008-9231-7]

59 **Velho S**, Fernandes MS, Leite M, Figueiredo C, Seruca R. Causes and consequences of microsatellite instability in gastric carcinogenesis. *World J Gastroenterol* 2014; **20**: 16433-16442 [PMID: 25469011 DOI: 10.3748/wjg.v20.i44.16433]

60 **Chung DC**, Rustgi AK. DNA mismatch repair and cancer. *Gastroenterology* 1995; **109**: 1685-1699 [PMID: 7557155]

61 Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 2012; **487**: 330-337 [PMID: 22810696 DOI: 10.1038/nature11252]

62 Correlation between mismatch repair deficiency (MMRd), microsatellite instability (MSI) and survival in MAGIC. *J Clin Oncol* [Internet]. [accessed 2016 Jul 28]. Available from: URL: http://meetinglibrary.asco.org/content/169920-176

63 **Pinto M**, Wu Y, Mensink RG, Cirnes L, Seruca R, Hofstra RM. Somatic mutations in mismatch repair genes in sporadic gastric carcinomas are not a cause but a consequence of the mutator phenotype. *Cancer Genet Cytogenet* 2008; **180**: 110-114 [PMID: 18206535 DOI: 10.1016/j.cancergencyto.2007.09.022]

64 **Elsaleh H**, Joseph D, Grieu F, Zeps N, Spry N, Iacopetta B. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000; **355**: 1745-1750 [PMID: 10832824 DOI: 10.1016/S0140-6736(00)02261-3]

65 **Zhu L**, Li Z, Wang Y, Zhang C, Liu Y, Qu X. Microsatellite instability and survival in gastric cancer: A systematic review and meta-analysis. *Mol Clin Oncol* 2015; **3**: 699-705 [PMID: 26137290 DOI: 10.3892/mco.2015.506]

66 **Bang YJ**, Im SA, Lee KW, Cho JY, Song EK, Lee KH, Kim YH, Park JO, Chun HG, Zang DY, Fielding A, Rowbottom J, Hodgson D, O'Connor MJ, Yin X, Kim WH. Randomized, Double-Blind Phase II Trial With Prospective Classification by ATM Protein Level to Evaluate the Efficacy and Tolerability of Olaparib Plus Paclitaxel in Patients With Recurrent or Metastatic Gastric Cancer. *J Clin Oncol* 2015; **33**: 3858-3865 [PMID: 26282658 DOI: 10.1200/JCO.2014.60.0320]

67 **Topalian SL**, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Sosman JA, Atkins MB, Leming PD, Spigel DR, Antonia SJ, Horn L, Drake CG, Pardoll DM, Chen L, Sharfman WH, Anders RA, Taube JM, McMiller TL, Xu H, Korman AJ, Jure-Kunkel M, Agrawal S, McDonald D, Kollia GD, Gupta A, Wigginton JM, Sznol M. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 2012; **366**: 2443-2454 [PMID: 22658127 DOI: 10.1056/NEJMoa1200690]

68 **Le DT**, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D, Biedrzycki B, Donehower RC, Zaheer A, Fisher GA, Crocenzi TS, Lee JJ, Duffy SM, Goldberg RM, de la Chapelle A, Koshiji M, Bhaijee F, Huebner T, Hruban RH, Wood LD, Cuka N, Pardoll DM, Papadopoulos N, Kinzler KW, Zhou S, Cornish TC, Taube JM, Anders RA, Eshleman JR, Vogelstein B, Diaz LA. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N Engl J Med* 2015; **372**: 2509-2520 [PMID: 26028255 DOI: 10.1056/NEJMoa1500596]

69 **Schwitalle Y**, Kloor M, Eiermann S, Linnebacher M, Kienle P, Knaebel HP, Tariverdian M, Benner A, von Knebel Doeberitz M. Immune response against frameshift-induced neopeptides in HNPCC patients and healthy HNPCC mutation carriers. *Gastroenterology* 2008; **134**: 988-997 [PMID: 18395080 DOI: 10.1053/j.gastro.2008.01.015]

70 **Van Cutsem E**, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda Aguilar E, Bardelli A, Benson A, Bodoky G, Ciardiello F, D'Hoore A, Diaz-Rubio E, Douillard JY, Ducreux M, Falcone A, Grothey A, Gruenberger T, Haustermans K, Heinemann V, Hoff P, Köhne CH, Labianca R, Laurent-Puig P, Ma B, Maughan T, Muro K, Normanno N, Österlund P, Oyen WJ, Papamichael D, Pentheroudakis G, Pfeiffer P, Price TJ, Punt C, Ricke J, Roth A, Salazar R, Scheithauer W, Schmoll HJ, Tabernero J, Taïeb J, Tejpar S, Wasan H, Yoshino T, Zaanan A, Arnold D. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol* 2016; **27**: 1386-1422 [PMID: 27380959 DOI: 10.1093/annonc/mdw235]

71 **Colli LM**, Machiela MJ, Myers TA, Jessop L, Yu K, Chanock SJ. Burden of Nonsynonymous Mutations among TCGA Cancers and Candidate Immune Checkpoint Inhibitor Responses. *Cancer Res* 2016; **76**: 3767-3772 [PMID: 27197178 DOI: 10.1158/0008-5472.CAN-16-0170]

72 **Samsung Medical Center**. Study of Pembrolizumab in Subjects With Advanced Gastric or Gastroesophageal Junction Adenocarcinoma Who Progressed After First-Line Therapy With Platinum and Fluoropyrimidine: Integration of Molecular Subtypes Through Integrative Genomic Analysis. In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jul 31]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT02589496 NLM Identifier: NCT02589496

73 **Muro K**, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, Eder JP, Golan T, Le DT, Burtness B, McRee AJ, Lin CC, Pathiraja K, Lunceford J, Emancipator K, Juco J, Koshiji M, Bang YJ.

Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial. *Lancet Oncol* 2016; **17**: 717-726 [PMID: 27157491 DOI: 10.1016/S1470-2045(16)00175-3]

74 **Bristol-Myers Squibb**. A Study of Nivolumab and Nivolumab Plus Ipilimumab in Recurrent and Metastatic Colon Cancer (CheckMate 142). In: ClinicalTrials.gov [Internet]. Bethesda (MD): National Library of Medicine (US). [accessed 2016 Jul 31]. Available from: URL: https://clinicaltrials.gov/ct2/show/NCT02060188 NLM Identifier: NCT02060188

75 **Buckowitz A**, Knaebel HP, Benner A, Bläker H, Gebert J, Kienle P, von Knebel Doeberitz M, Kloor M. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. *Br J Cancer* 2005; **92**: 1746-1753 [PMID: 15856045 DOI: 10.1038/sj.bjc.6602534]

76 **Corso G**, Marrelli D, Pascale V, Vindigni C, Roviello F. Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. *BMC Cancer* 2012; **12**: 8 [PMID: 22225527 DOI: 10.1186/1471-2407-12-8]

77 **Liu YC**, Shen CY, Wu HS, Hsieh TY, Chan DC, Chen CJ, Yu JC, Yu CP, Harn HJ, Chen PJ, Hsieh CB, Chen TW, Hsu HM. Mechanisms inactivating the gene for E-cadherin in sporadic gastric carcinomas. *World J Gastroenterol* 2006; **12**: 2168-2173 [PMID: 16610016]

78 **Cavallaro U**, Christofori G. Cell adhesion and signalling by cadherins and Ig-CAMs in cancer. *Nat Rev Cancer* 2004; **4**: 118-132 [PMID: 14964308 DOI: 10.1038/nrc1276]

79 **Li X**, Wu WK, Xing R, Wong SH, Liu Y, Fang X, Zhang Y, Wang M, Wang J, Li L, Zhou Y, Tang S, Peng S, Qiu K, Chen L, Chen K, Yang H, Zhang W, Chan MT, Lu Y, Sung JJ, Yu J. Distinct Subtypes of Gastric Cancer Defined by Molecular Characterization Include Novel Mutational Signatures with Prognostic Capability. *Cancer Res* 2016; **76**: 1724-1732 [PMID: 26857262 DOI: 10.1158/0008-5472.CAN-15-2443]

80 **Weissman B**, Knudsen KE. Hijacking the chromatin remodeling machinery: impact of SWI/SNF perturbations in cancer. *Cancer Res* 2009; **69**: 8223-8230 [PMID: 19843852 DOI: 10.1158/0008-5472.CAN-09-2166]

81 **Wang DD**, Chen YB, Pan K, Wang W, Chen SP, Chen JG, Zhao JJ, Lv L, Pan QZ, Li YQ, Wang QJ, Huang LX, Ke ML, He J, Xia JC. Decreased expression of the ARID1A gene is associated with poor prognosis in primary gastric cancer. *PLoS One* 2012; **7**: e40364 [PMID: 22808142 DOI: 10.1371/journal.pone.0040364]

82 **Van Aelst L**, D'Souza-Schorey C. Rho GTPases and signaling networks. *Genes Dev* 1997; **11**: 2295-2322 [PMID: 9308960]

83 **Hall A**. Rho GTPases and the actin cytoskeleton. *Science* 1998; **279**: 509-514 [PMID: 9438836]

84 **Aznar S**, Valerón PF, del Rincon SV, Pérez LF, Perona R, Lacal JC. Simultaneous tyrosine and serine phosphorylation of STAT3 transcription factor is involved in Rho A GTPase oncogenic transformation. *Mol Biol Cell* 2001; **12**: 3282-3294 [PMID: 11598209]

85 **Thumkeo D**, Watanabe S, Narumiya S. Physiological roles of Rho and Rho effectors in mammals. *Eur J Cell Biol* 2013; **92**: 303-315 [PMID: 24183240 DOI: 10.1016/j.ejcb.2013.09.002]

86 **Narumiya S**, Tanji M, Ishizaki T. Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. *Cancer Metastasis Rev* 2009; **28**: 65-76 [PMID: 19160018 DOI: 10.1007/s10555-008-9170-7]

87 **Kakiuchi M**, Nishizawa T, Ueda H, Gotoh K, Tanaka A, Hayashi A, Yamamoto S, Tatsuno K, Katoh H, Watanabe Y, Ichimura T, Ushiku T, Funahashi S, Tateishi K, Wada I, Shimizu N, Nomura S, Koike K, Seto Y, Fukayama M, Aburatani H, Ishikawa S. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet* 2014; **46**: 583-587 [PMID: 24816255 DOI: 10.1038/ng.2984]

88 **Doherty GJ**, Åhlund MK, Howes MT, Morén B, Parton RG, McMahon HT, Lundmark R. The endocytic protein GRAF1 is directed to cell-matrix adhesion sites and regulates cell spreading. *Mol Biol Cell* 2011; **22**: 4380-4389 [PMID: 21965292 DOI: 10.1091/mbc.E10-12-0936]

89 **Türeci O**, Koslowski M, Helftenbein G, Castle J, Rohde C, Dhaene K, Seitz G, Sahin U. Claudin-18 gene structure, regulation, and expression is evolutionary conserved in mammals. *Gene* 2011; **481**: 83-92 [PMID: 21571049 DOI: 10.1016/j.gene.2011.04.007]

90 **Yao F**, Kausalya JP, Sia YY, Teo AS, Lee WH, Ong AG, Zhang Z, Tan JH, Li G, Bertrand D, Liu X, Poh HM, Guan P, Zhu F, Pathiraja TN, Ariyaratne PN, Rao J, Woo XY, Cai S, Mulawadi FH, Poh WT, Veeravalli L, Chan CS, Lim SS, Leong ST, Neo SC, Choi PS, Chew EG, Nagarajan N, Jacques PÉ, So JB, Ruan X, Yeoh KG, Tan P, Sung WK, Hunziker W, Ruan Y, Hillmer AM. Recurrent Fusion Genes in Gastric Cancer: CLDN18-ARHGAP26 Induces Loss of Epithelial Integrity. *Cell Rep* 2015; **12**: 272-285 [PMID: 26146084 DOI: 10.1016/j.celrep.2015.06.020]

91 FAST: An international, multicenter, randomized, phase II trial of epirubicin, oxaliplatin, and capecitabine (EOX) with or without IMAB362, a first-in-class anti-CLDN18.2 antibody, as first-line therapy in patients with advanced CLDN18.2 gastric and gastroesophageal junction (GEJ) adenocarcinoma. *J Clin Oncol* [Internet]. [accessed 2016 Jul 31]. Available from: URL: http://meetinglibrary.asco.org/content/164788-176

92 **Guan R**, Xu X, Chen M, Hu H, Ge H, Wen S, Zhou S, Pi R. Advances in the studies of roles of Rho/Rho-kinase in diseases and the development of its inhibitors. *Eur J Med Chem* 2013; **70**: 613-622 [PMID: 24211637 DOI: 10.1016/j.ejmech.2013.10.048]

93 **Manfredi MG**, Ecsedy JA, Chakravarty A, Silverman L, Zhang M, Hoar KM, Stroud SG, Chen W, Shinde V, Huck JJ, Wysong DR, Janowick DA, Hyer ML, Leroy PJ, Gershman RE, Silva MD, Germanos MS, Bolen JB, Claiborne CF, Sells TB. Characterization of Alisertib (MLN8237), an investigational small-molecule inhibitor of aurora A kinase using novel in vivo pharmacodynamic assays. *Clin Cancer Res* 2011; **17**: 7614-7624 [PMID: 22016509 DOI: 10.1158/1078-0432.CCR-11-1536]

94 **Strebhardt K**, Ullrich A. Targeting polo-like kinase 1 for cancer therapy. *Nat Rev Cancer* 2006; **6**: 321-330 [PMID: 16557283 DOI: 10.1038/nrc1841]

95 **Hofheinz RD**, Al-Batran SE, Hochhaus A, Jäger E, Reichardt VL, Fritsch H, Trommeshauser D, Munzert G. An open-label, phase I study of the polo-like kinase-1 inhibitor, BI 2536, in patients with advanced solid tumors. *Clin Cancer Res* 2010; **16**: 4666-4674 [PMID: 20682708 DOI: 10.1158/1078-0432.CCR-10-0318]

96 **Schöffski P**, Awada A, Dumez H, Gil T, Bartholomeus S, Wolter P, Taton M, Fritsch H, Glomb P, Munzert G. A phase I, dose-escalation study of the novel Polo-like kinase inhibitor volasertib (BI 6727) in patients with advanced solid tumours. *Eur J Cancer* 2012; **48**: 179-186 [PMID: 22119200 DOI: 10.1016/j.ejca.2011.11.001]

97 **Bae JM**, Kim EH. Epstein-Barr Virus and Gastric Cancer Risk: A Meta-analysis With Meta-regression of Case-control Studies. *J Prev Med Public Health* 2016; **49**: 97-107 [PMID: 27055546 DOI: 10.3961/jpmph.15.068]

98 **Murphy G**, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of Epstein-Barr virus-positive gastric cancer differs based on sex and anatomic location. *Gastroenterology* 2009; **137**: 824-833 [PMID: 19445939 DOI: 10.1053/j.gastro.2009.05.001]

99 **Camargo MC**, Murphy G, Koriyama C, Pfeiffer RM, Kim WH, Herrera-Goepfert R, Corvalan AH, Carrascal E, Abdirad A, Anwar M, Hao Z, Kattoor J, Yoshiwara-Wakabayashi E, Eizuru Y, Rabkin CS, Akiba S. Determinants of Epstein-Barr virus-positive gastric cancer: an international pooled analysis. *Br J Cancer* 2011; **105**: 38-43 [PMID: 21654677 DOI: 10.1038/bjc.2011.215]

100 **Kim SY**, Park C, Kim HJ, Park J, Hwang J, Kim JI, Choi MG, Kim S, Kim KM, Kang MS. Deregulation of immune response genes in patients with Epstein-Barr virus-associated gastric cancer and outcomes. *Gastroenterology* 2015; **148**: 137-147.e9 [PMID: 25254613 DOI: 10.1053/j.gastro.2014.09.020]

101 **Derks S**, Liao X, Chiaravalli AM, Xu X, Camargo MC, Solcia E, Sessa F, Fleitas T, Freeman GJ, Rodig SJ, Rabkin CS, Bass AJ. Abundant PD-L1 expression in Epstein-Barr Virus-infected gastric

cancers. *Oncotarget* 2016; **7**: 32925-32932 [PMID: 27147580 DOI: 10.18632/oncotarget.9076]

102 **Dong M**, Wang HY, Zhao XX, Chen JN, Zhang YW, Huang Y, Xue L, Li HG, Du H, Wu XY, Shao CK. Expression and prognostic roles of PIK3CA, JAK2, PD-L1, and PD-L2 in Epstein-Barr virus-associated gastric carcinoma. *Hum Pathol* 2016; **53**: 25-34 [PMID: 26980034 DOI: 10.1016/j.humpath.2016.02.007]

103 **Frampton GM**, Ali SM, Rosenzweig M, Chmielecki J, Lu X, Bauer TM, Akimov M, Bufill JA, Lee C, Jentz D, Hoover R, Ou SH, Salgia R, Brennan T, Chalmers ZR, Jaeger S, Huang A, Elvin JA, Erlich R, Fichtenholtz A, Gowen KA, Greenbowe J, Johnson A, Khaira D, McMahon C, Sanford EM, Roels S, White J, Greshock J, Schlegel R, Lipson D, Yelensky R, Morosini D, Ross JS, Collisson E, Peters M, Stephens PJ, Miller VA. Activation of MET via diverse exon 14 splicing alterations occurs in multiple tumor types and confers clinical sensitivity to MET inhibitors. *Cancer Discov* 2015; **5**: 850-859 [PMID: 25971938 DOI: 10.1158/2159-8290.CD-15-0285]

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