Cancer Treatment Reviews 77 (2019) 29–34

Contents lists available at ScienceDirect

Cancer Treatment Reviews

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

Tumour Review

Comparison and applicability of molecular classifications for gastric cancer

O. Serra[a,c,f,*], M. Galán[c,f], M.M. Ginesta[b,c,e], M. Calvo[a,c,f], N. Sala[d,1], R. Salazar[a,c,e,1]

[a] Department of Medical Oncology, Catalan Institute of Oncology (ICO), Hospital Duran y Reynals, Hospitalet de Llobregat, Barcelona, Spain
[b] Translational Research Laboratory, Catalan Insitute of Oncology (ICO), Hospital Duran y Reynals, Hospitalet de Llobregat, Barcelona, Spain
[c] Oncobell Program, Bellvitge Biomedical Research Institute (IDIBELL) – University of Barcelona, Hospital Duran y Reynals, Hospitalet de Llobregat, Barcelona, Spain
[d] Unit of Nutrition and Cancer, Cancer Epidemiology Research Program and Translational Research Laboratory, Catalan Institute of Oncology (ICO) – Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain
[e] CIBERONC Centro de Investigación Biomédica en Red Cáncer, Spain
[f] Gastroesophageal Tumors Functional Unit (UTEG), Catalan Institute of Oncology (ICO) – Hospital Universitari de Bellvitge (HUB), Hospitalet de Llobregat, Barcelona, Spain

ARTICLE INFO

Keywords:
Gastric Cancer (GC)
Molecular classification
The Cancer Genome Atlas (TCGA)
Asian Cancer Research Group (ACRG)
Singapore-Duke
GC subtypes

ABSTRACT

Gastric Cancer (GC) is a complex and heterogeneous disease, which represents a global health concern. Despite advances in prevention, diagnosis, and therapy, GC is still a leading cause of cancer-related death. Over the last decade, several clinical trials have tested novel agents for advanced GC with mostly disappointing results. Heterogeneity, the absence of molecular selection in clinical trials and powerless predictive biomarkers may be potential explanations. Different molecular classification proposals for GC based on the genetic, epigenetic, and molecular signatures have been published. Molecular characterization of GC may offer new tools for more effective therapeutic strategies, such as the development of therapies for specifically well-defined sets of patients as well as the use of new clinical trial designs, which will ultimately lead to an improvement of medical management of this disease. However, the possibilities of implementation of GC molecular classifications on daily practice and their therapeutic implications remain challenging to date. In this review, we will describe and compare these GC molecular classifications, focusing on their main characteristics as the basis for their potential therapeutic implications and strategies for their clinical application.

Key Message: A better understanding of gastric cancer molecular characteristics may lead to further improvements in treatment and outcomes for patients with the disease.

Introduction

GC is a heterogeneous disease, which currently ranks as the fifth most common cancer and the third leading cause of cancer-related death worldwide [1]. Despite many efforts to improve treatment strategies in the last decade, gastric cancer (GC) still presents dismal outcomes. Only 30% of GC are diagnosed at a local stage and the 5-year survival for pTNM stage groups classified according to the 8th edition of the Cancer staging manual of the American Joint Committee on Cancer (AJCC) are between 80 and 68% for stage I, 60 and 46% for stage II, 30 and 8% for stage III and 5% for stage IV, based on data from NCDB (National Cancer Database) Research Unit of the American College of Surgeons [2].

The complexity and heterogeneity of GC emerge from multiple interactions of genetic, environmental and host factors [3,4]. Historically, epidemiologic and histopathologic differences, as well as disparities in survival rates, have been reported in patients diagnosed with GC from different regions, especially between Asian and non-Asian countries [5,6]. More recently, different immunity signatures between Asian and non-Asian population have also been described [7]. Whilst genetic, environmental and other host factors may account for some of these differences, the interpretation of different survival outcomes is even more complicated due to variations in the staging systems, extent and quality of surgery and heterogeneity of adjuvant and neoadjuvant treatment regimens between Asian and Western countries.

GC has traditionally been classified into two major histological subtypes according to Lauren's classification: intestinal and diffuse [8]. Diffuse-type gastric adenocarcinoma is more aggressive, tends to affect younger people and does not progress through distinct histological stages, whereas intestinal-type gastric adenocarcinoma is characterized

* Corresponding author at: Oncology Department – Gastrointestinal Cancer Unit, Catalan Institute of Oncology – IDIBELL – University of Barcelona, Hospital Duran y Reynals, Avenida de la Granvia 199-203, Hospitalet de Llobregat, 08908 Barcelona, Spain.
E-mail address: oserra@iconcologia.net (O. Serra).
[1] These authors share co-senior authorship.

https://doi.org/10.1016/j.ctrv.2019.05.005
Received 4 March 2019; Received in revised form 20 May 2019; Accepted 21 May 2019

by histological progression from *Helicobacter pylori*-associated inflammatory cell invasion to atrophic gastritis, intestinal metaplasia, dysplasia and ultimately adenocarcinoma [4]. Apart from Lauren's classification, other histopathological classifications for GC exist, such as the World Health Organization (WHO) classification, which divides GC into four main types based on the predominant histological patterns: tubular adenocarcinoma, papillary adenocarcinoma, mucinous adenocarcinoma and poorly cohesive carcinoma [9]. Among these types, the poorly cohesive carcinoma would correspond to the diffuse-type and the tubular and papillary carcinomas would correspond to the intestinal-type. These histopathological classifications are widely used, but they allow us neither to select patients who can benefit from a specific chemotherapy or novel therapies, nor to precisely predict prognosis. The description of different molecular classifications of GC during the last years, among which that of The Cancer Genome Atlas (TCGA) [10], could be more helpful in clinical practice.

With regard to medical management, several new agents for the treatment of advanced GC have been investigated in the last years, and many good reviews on potential new treatments have been published [11]. Studies in different disease settings with antiangiogenics, anti-EGFR or anti-MET monoclonal antibodies have been negative. In contrast, trastuzumab, a monoclonal antibody against the HER2 receptor, is approved in the first line treatment of patients with HER2 + tumors, which accounts for 13–23% of the gastric cancer population [12]. Ramucirumab, a monoclonal antibody against VEGFR2, is currently recommended in patients progressing after first-line treatment based on fluoropyrimidine and platinum [13,14]. Likewise, apatinib, a tyrosine kinase inhibitor (TKI) that selectively inhibits VEGFR2, has also been approved in China in the same setting. Besides, emerging data from early trials suggest that the use of immune checkpoint inhibitors may result in durable remissions for a proportion of patients with advanced GC [15,16].

The purpose of the present review is two-fold: first, to describe the main molecular classifications of GC, and compare them to find differences and similarities between the different subgroups. Second, we want to define their therapeutic implications and potential clinical applicability in daily medical practice.

Molecular classifications of GC

Initial studies to describe molecular GC subtypes were reported by the "Singapore – Duke" Group [17,18]. These were followed by large-scale efforts from the Asian Cancer Research Group (ACRG) [19] and The Cancer Genome Atlas (TCGA) [20].

"Singapore – Duke" group proposal

Singapore-Duke Group was the first to describe GC molecular subtypes. They used gene expression profiling to predict GC response to drug treatments. Initially, they described a classification that divided GC into two intrinsic genomic subtypes that had distinct patterns of gene expression: G-INT (genomic intestinal) and G-DIF (genomic diffuse). Although they named them G-INT and G-DIF due to similarities with Lauren's histopathologic classification, the overall concordance between these intrinsic subtypes and the Lauren's histopathologic classification was only 64%. Compared with the G-DIF subtype, G-INT subtype showed higher resistance to cisplatin, but sensitivity to 5-fluorouracil and oxaliplatin. Interestingly, these two intrinsic subtypes, as opposed to the subtypes based on Lauren's histopathologic classification, have been shown to have prognostic value, based on univariate and multivariate analyses in multiple patient cohorts [17].

Subsequently, the Singapore-Duke group reported a new proposal that classified GC into three different subtypes: proliferative, metabolic and mesenchymal [18]. The proliferative subtype, which was found to be associated with the Lauren intestinal subtype, presented with high activity for several oncogenic pathways and was characterized by increased *TP53* mutations and high copy number alterations (CNA). The mesenchymal subtype was associated with the Lauren diffuse subtype and was characterized by high activity of the epithelial-mesenchymal transition pathway as well as cancer stem cell pathways. A critical issue of this new proposal is the emergence of metabolic subtype as a new entity which showed high activity for a pathway related to spasmolytic-polypeptide-expressing metaplasia (SPEM) [21,22]. Lei et al. [18] described differences in response to therapy between proliferative, metabolic and mesenchymal subtypes. *In vitro* studies indicated that mesenchymal subtype was particularly sensitive to phosphatidylinositol 3-kinase (PI3K)-AKT-mTOR inhibitors and the metabolic subtype was more sensitive to 5-fluorouracil.

Asian Cancer Research group (ACRG) proposal

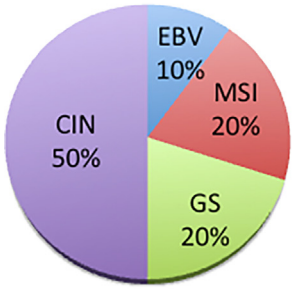
The Asian Cancer Research Group (ACRG) categorizes GC into four subtypes by applying gene expression data [19]. These subtypes are: microsatellite instability (MSI), epithelial-to-mesenchymal transition (EMT), MSS/TP53 + and MSS/TP53-. MSI group was characterized by loss of *MLH1* and was enriched by mutations in several oncogenic genes. The microsatellite stable (MSS) and EMT group (MSS/EMT) was characterized by loss of *CDH1* and a gene expression signature that correlated with that of EMT. Since *TP53* is the most frequently mutated gene in GC, the non-MSI and non-EMT tumors were grouped into MSS/TP53 + (TP53-active) and MSS/TP53- (TP53-inactive). This was based on a two-gene p53-activity signature (*CDKN1A* and *MDM2*) that scored high in tumors with intact TP53 and low in tumors with functional loss of TP53. The cohort of TP53-inactive samples presented the highest prevalence of *TP53* mutations, had higher aneuploidy and more amplifications of oncogenes, including *ERBB2* (HER2). Infection with Epstein-Barr virus (EBV) was more frequent in the MSS/TP53 + group. The subgroups described by ACRG were found to be associated with distinct genomic alterations, survival outcomes and recurrence patterns after surgery [19]. In the survival analysis, the EMT group (which was composed mostly of diffuse-type tumors) had the worst prognosis, followed by TP53-inactive, TP53-active and finally microsatellite-unstable tumors.

The Cancer Genome Atlas (TCGA) proposal

The Cancer Genome Atlas (TCGA) research network reported the most comprehensive identification of genetic alterations associated with GC, combining data from six different platforms: array-based somatic copy number analysis, whole-exome and genome sequencing, messenger RNA-sequencing, microRNA sequencing, and reverse-phase protein array profiling, plus evaluation of microsatellite instability [20]. Based on an integrative analysis of these data, a classification system of GC into four subtypes was proposed: EBV-positive (EBV), microsatellite-unstable (MSI), genomically stable (GS) and chromosomal instability (CIN).

EBV-positive (EBV) tumors presented extensive DNA promoter hypermethylation, as well as recurrent *PIK3CA* mutations and amplifications of *JAK2*, *CD274* (also known as PD-L1) and *PDCD1LG2* (also known as PD-L2); *ARID1A* mutations were also present in more than 50% of EBV-positive tumors which, by contrast, had few *TP53* mutations. Microsatellite-unstable tumors (MSI) showed high mutation burden and hypermethylation, particularly that of the *MLH1* promoter. Genomically stable tumors (GS) were enriched for Lauren's diffuse histological type of GC and showed *RHOA* mutations or fusions involving Rho-family GTPase-activating proteins; *CDH1* and *ARID1A* somatic mutations were also common in the GS subtype. Finally, tumors with chromosomal instability (CIN) had marked aneuploidy and focal amplification of receptor tyrosine kinases and were enriched in *TP53* mutations. As illustrated in Fig. 1, this classification also provides information about potential therapeutic implications and drug resistance.

In the TCGA study no analysis of survival outcome was performed.



TCGA Subtype	Molecular Alterations	Potential Therapeutic Implications
CIN	Amplifications of RTK genes	***Targeted therapy according to each alteration***
	Amplifications of cell cycle genes	***CDK inhibitors***
	Amplifications of VEGF gene	***Antiangiogenic therapy***
EBV	PI3KCA mutations	***PI3KCA inhibitors / resistance to upstream inhibitors*** Ref. [22]
	Overexpression of JAK2	***JAK2 inhibitors***
	Overexpression of PD-1/L1	***Anti-PD-1/L1 antibodies***
	DNA hypermethylation status	***DNA hypomethylating agents ?***
	ARID1A mutations	***Anti-PD-1/L1 antibodies*** Ref. [25]
MSI	Hypermutation status	***Anti-PD-1/L1 antibodies***
	PI3KCA mutations	***PI3KCA inhibitors / resistance to upstream inhibitors*** Ref. [22]
	ERBB1-3 mutations	***HER2-targeted drugs / resistance to antiEGFR or HER2*** if HER3 mutation Ref. [23]
	Microsatellite Instability	**5-fluorouracil-resistance** Ref. [24]
GS	Elevated expression of mitotic, cell adhesion and angiogenesis-related pathways	***AURKA/B*** *or* ***PLK inhibitors or antiangiogenic therapy***
	Mutations in ARID1A, CLDN18, CDH1 and RHOA and amplifications of FGFR2	***Anti-PD-1/L1 antibodies*** if ARID1A mutation Ref. [25], **FGFR2 inhibitors** or other potential treatments to be defined such as anti-claudin antibodies

Fig. 1. Gastric cancer molecular subtypes. (A) Distribution of gastric cancer molecular subtypes by TCGA. (B) Main molecular features of each subtype and their potential therapeutic implications according to the TCGA report. Potential drug resistance explained by the same molecular alterations are also indicated. *CIN* chromosomal instability, *EBV* Epstein-Barr virus, *MSI* microsatellite instable, *GS* genomically stable. AURKA/B Aurora kinase, *PLK* polo-like kinase, *CDK* Cyclin-dependent kinase, *RTK* receptor tyrosine kinase, *FGFR2* fibroblast growth factor receptor 2, *VEGF* vascular endothelial growth factor, *PD-L1/L1* programmed cell death ligand, *JAK2* janus kinase 2, *PI3KCA* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha, *ERBB1-3* receptor tyrosine-protein kinase erbB1-3, *HER2* human epidermal growth factor receptor 2, *ARID1A* AT-rich interactive domain-containing protein 1A, *CLDN18* Claudin18, *CDH1* cadherin1 and *RHOA* Ras homolog gene family member A, *FGFR2* fibroblast growth factor receptor 2.

Afterwards, there have been some reports showing that EBV-positive and MSI GC have a better prognosis [23,24]. In addition, Li et al. [25] have recently published a new classification of GC based on mutation burden and mutation patterns, identifying some of the subgroups as predictors of patient survival.

Comparison between molecular classifications: Similarities and differences

The previously described substantial efforts to molecularly characterize GC have some overlapping, but also distinct features which are discussed in more detail in this section.

Type of analysis and samples

DNA somatic mutation and MSI characterization were not available in the Singapore-Duke set. The molecular analysis in TCGA and ACRG sets was broader. Singapore-Duke and ACRG classifications were based on mRNA expression that in ACRG was complemented with genome-wide copy number data and targeted gene sequencing, whereas TCGA analysis was based on an integration of six distinct molecular platforms. TCGA and ACRG analyzed surgical specimens of primary tumors that had not previously been treated with chemotherapy or radiotherapy, whereas the Singapore-Duke cohort analysis was based on gastric cancer cell lines, which may have prevented from capturing the cellular heterogeneity of the tumor.

Data collection

The ACRG study accrued patients from a single referral hospital in South Korea, with valuable clinical information available, while the TCGA study collected samples from several institutions around the world without data on survival outcomes or recurrence patterns after surgery. Thus, the ACRG study represents a more homogeneously treated cohort.

Table 1 shows the main patient characteristics and relevant information about data collection and analysis in the TCGA and ACRG cohorts. This table does not include information from the Singapore-Duke study due to lack of availability of some data.

Table 1
Main characteristics of the analyzed patients, tumor samples and type of analysis in the TCGA and ACRG studies.

	TCGA	ACRG
Ethnicity	25% East Asian	100% Korean
Histology	23% diffuse	45% diffuse
Stage	31% III/IV	57% III/IV
Location	19% GEJ	11% GEJ
Type of sample	Primary tumor	Primary tumor
Molecular analysis	Comprehensive	More limited
Clinical data	Limited	Extensive
Survival data	No survival analysis	Long-term follow up-data
Accrual	Several centers	Single referral hospital

TCGA The Cancer Genome Atlas, *ACRG* Asian Cancer Research Group, *GEJ* gastroesophageal junction.

Resulting molecular subgroups and equivalences between molecular subtypes

The TCGA and ACRG projects developed a four-group classification for GC, whereas the Singapore-Duke study divided GC in a three-group classification. Microsatellite instability (MSI) was identified as a subgroup by the TCGA as well as the ACRG studies, but not by the Singapore-Duke study. However, in TCGA, the EBV-positive tumors emerged as a distinct entity. GS from TCGA and MSS/EMT from ACRG were subgroups enriched by Lauren's diffuse subtype. In turn, CIN from TCGA and MSS/TP53- from ACRG were subgroups with marked aneuploidy and focal amplification of receptor tyrosine kinases.

Therefore, two questions arise:

1. Is the GS group from TCGA similar to the EMT group from ACRG?

Although both GS and EMT tumors were mostly of the diffuse type (75% and 80% respectively), the distribution of diffuse type in the other subgroups is different in TCGA and ACRG cohorts. There are more diffuse tumors among non-EMT subgroups in the ACRG set (30–46%) than among non-GS subgroups in the TCGA set (< 25%) [19,20]. Moreover, *CDH1* mutations were highly prevalent in the TCGA GS subgroup (37%), but they were infrequent in the ACRG EMT subgroup (2.8%). *RHOA* mutations, characteristic of the TCGA GS subgroup, were also infrequent in ACRG EMT subgroup being more prevalent in other ACRG subgroups. Kwon et al. [26] have recently described the lack of RHOA mutations in signet ring cell -predominant tumors in comparison to other types of poorly cohesive carcinomas.

2. Are the CIN group from TCGA and the TP53-inactive (MSS/TP53-) group from ACRG, the same?

Both CIN and MSS/TP53- subgroups have a high degree of structural genomic alterations, leading to a high frequency of genomic amplifications. The CIN subgroup is much more frequent than the MSS/TP53- subgroup. This difference may reflect not only the different classification system but also differences in the populations studied. CIN tumors were more prevalent in the proximal stomach, and the incidence of proximal GC cases in Western populations has risen in recent years. Furthermore, in the ACRG cohort, the percentage of Lauren's diffuse subtype was higher, and the frequency of proximal GC was lower, than in the TCGA study.

Thus, there is no precise correspondence of Lauren's diffuse type to GS or EMT subgroups. In addition, despite some overlap, the differences related to *CDH1* and *RHOA* mutations suggest that GS and EMT subgroups are not the same. CIN subgroup and MSS/TP53- match quite closely by sharing a high degree of structural genomic aberrations as a crucial feature. The distribution of TCGA gastric data set tumors using ACRG subtypes and compared to TCGA subtypes shows that the correspondence between GS and EMT as well as between CIN and MSS/TP53- was about 50% and 85%, respectively [27]. Despite difficulties in comparing the different classifications due to differences in the populations analyzed and the molecular analyses performed, equivalences between them are shown in Table 2.

Predictive value of molecular classifications

Sohn et al. [28] have recently published the validation of a model for subtype allocation, based on gene expression data from the TCGA cohortin two independent cohorts. They retrospectively analyzed the association of each subtype with both, survival and benefit from adjuvant chemotherapy. The prognosis analysis is similar to previous results. Patients with the CIN subtype derived the most significant benefit from adjuvant chemotherapy and those with the GS subtype had the least benefit from adjuvant chemotherapy. However, the retrospective design of the study and the non-randomized assignment to adjuvant chemotherapy, limits the interpretation of these results.

Table 2
Equivalences between gastric cancer molecular subtypes among the different proposals of molecular classifications.

Study	Equivalent molecular subtypes				
Singapore-Duke	*Mesenchymal* (Lauren's diffuse type)	*Proliferative* (Lauren's intestinal type)	*Metabolic*	--	--
ACRG	*MSS/EMT*	*MSS/TP53-*	**MSS/TP53+*	*MSI*	--
TCGA	*GS*	*CIN*	--	*MSI*	*EBV*
Setia/Ahn	*Aberrant E-cadherin*	*Aberrant TP53*	*Normal TP53*	*MSI-H*	*EBV*

In pink approximative equivalences. In grey cost-effective classifications. (*) In ACRG cohort, EBV infection occurred more frequently in the MSS/TP53+ group than in the other groups.
CIN chromosomal instability, *EBV* Epstein-Barr virus, *MSI* microsatellite instable, *GS* genomically stable, *MSS* Microsatellite Stable, *EMT* epithelial-to-mesenchymal transition, *TP53−* *TP53 inactive, TP53+* TP53 active, *MSI-H* MSI-high status.

Moreover, recently published results from an exploratory analysis of the MAGIC trial [29] show that patients with operable gastro-esophageal cancer with MSI-low or MSS tumors experienced improved survival compared with patients with MSI-high tumors when treated with perioperative chemotherapy plus surgery, which suggests that high-MSI subgroup does not benefit from perioperative chemotherapy. Analysis of the effect of MSI on survival in the CLASSIC trial showed a similar lack of benefit from adjuvant chemotherapy in this subset of patients [30]. Nevertheless, independent validation of these results is needed to know if MSI or mismatch repair (MMR) deficiency (MMRD) could help for treatment decision-making. In the advanced setting, Kim et al. reported dramatic responses to anti-PD-1 therapy for MSI and EBV positive tumors, with 85% and 100% overall response rates, respectively [31].

Molecular classifications in routine practice: Cost-effective classifications

The identification of different molecular subtypes has expanded our insight into the complex nature of GC and may provide useful information for treatment decisions in the future. Nevertheless, the cost and complexity of the technologies used in these classifications are still significant obstacles to their practical application.

Two investigator teams have recently published their proposals for molecular classifications of GC using more straightforward techniques, which may facilitate their application on daily practice. Setia et al. [32] reported a protein and mRNA expression-based molecular classification of GC in a cohort of Western patients (n = 146), using widely available techniques of immunohistochemistry (IHC) and in situ hybridization (ISH). Five subgroups were identified with relevance to clinicopathologic features and trends in clinical outcome: EBV (Epstein-Barr virus) tumors, MSI-H tumors, aberrant E-cadherin tumors (GC with aberrant E-cadherin expression), aberrant p53 tumors (GC with aberrant p53 expression), and normal p53 (GC with normal p53 expression). As shown in Table 2, Setia classification results in an interesting combination of the three large-scale efforts of molecular classifications in GC. The results of this study were validated by Ahn et al. [33] in a large-scale Asian cohort (n = 349), in which substantial differences in overall survival were observed between these five subtypes.

Setia and Ahn studies confirm previously known geographical differences in subtype prevalences between Asian and Western cohorts. For example, prevalence of MSI GC was higher in Western (16%) than in Asian population (6.9%). Furthermore, both studies confirm that a molecular classification of GC can be achieved using commercially available biomarkers and Tissue Microarray (TMA) technology showing a proper correspondence with survival analysis in TCGA and ACRG projects.

Despite not yet developed, an alternative to perform a molecular classification in a routine clinical setting could be the use of qRT-PCR to determine the expression of a selected group of genes representative of the subtype prediction model, as the ones described by Sohn et al. [28].

Limitations of cost-effective classifications

The use of TMA from endoscopic biopsy material, which is usually the only diagnostic source in routine practice, is relatively unexplored and only a few groups have used this approach [34]. A limitation of this technique is that the small size of the tissue sample used in a TMA (0.6–1 mm core biopsies) cannot be representative of the actual range of intratumor and intrapatient heterogeneous protein expression in GC. All major molecular classifications have been developed based on analyses of primary tumors, which may not reflect the real scenario of advanced disease [35]. Recently, Pectasides et al. have reported a high level of discordance, up to 87% for targetable alterations, between primary and metastatic lesions [36]. The lower prevalence of HER2 overexpression observed by Setia (2.3%) [32], and Ahn (3.2%) [33] as compared to the screening data from the ToGA trial (22%) [12] may be attributed to this limitation. Moreover, some subtypes such as MSI and EBV account for a much lower frequency in patients with advanced disease than in non-advanced disease, because of their inherent positive prognostic value. Finally, the biomarkers used in the IHC/ISH panels may not always reflect the complexity of the molecular alterations underlying an altered gene or protein expression and may not correctly distribute all samples over the different GC subtypes. For instance, it has been shown that altered *E-cadherin* expression by IHC is quite often not related to *CDH1* molecular alterations but to alterations in other regulatory mechanisms [37].

Future directions and therapeutic implications

Based on the potential therapeutic implications, testing for MSI and EBV may be informative in routine practice. Further improvement in predictive and therapeutic biomarkers for other subtypes is still required for a better GC stratification. For instance, in CIN subtype, a molecular group enriched by a wide range of tyrosine kinase receptor amplifications, a refinement of biomarkers is required, and future studies should not be focused on a unique biomarker in these tumors. A better characterization of molecular subtypes can guide us in the selection of more specific biomarkers for each subtype.

Methods for GC stratification based on protein and mRNA expression by IHC/ISH or qRT-PCR analysis could be a useful and cost-effective tool in daily practice. However, further development of the prediction model is warranted, and more validations are required, especially in non-Asian populations. The addition of next-generation sequencing analysis of specific gene panels covering the most common and confirmed actionable gene alterations in the different molecular subtypes of GC may help to overcome the limitations of these IHC/ISH methods [38,39].

To conclude, emerging data from molecular classifications is powerful and promising. All this new information should help in the design of future clinical trials and in the development of new drugs for tailored therapies in GC, improving patient outcomes. Furthermore, it should also help to redefine biomarkers to distinguish those patients who would benefit from specific treatments from those who would not. This would increase cost-efficiency of the newly developed drugs and would avoid unnecessary toxicities.

Funding

This work was supported by Health Research Funds from 'Instituto de Salud Carlos III', Spanish Ministry of Health, Spain, cofounded by European Regional Development Funds (ERDF/FEDER) '*A way to build Europe*', http://www.isciii.es/, grant number FIS Exp. PI16/00599 to Dr. N Sala and Dr. M Galan. We also acknowledge support from Bellvitge Biomedical Research Institute (IDIBELL), CERCA Programme for research centres of Catalonia, Generalitat de Catalunya and the '*Viladecans contra el càncer*' association.

Declaration of Competing Interest

The authors have declared no conflicts of interest.

References

[1] Ferlay J, Colombet M, Soerjomataram I, Mathers C, Parkin DM, Piñeros M, et al. Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods Dec 6 [cited 2019 Apr 12]. Available from:. Int J Cancer [Internet] 2018;144(8):ijc.31937http://www.ncbi.nlm.nih.gov/pubmed/30350310.

[2] In H, Solsky I, Palis B, Langdon-Embry M, Ajani J, Sano T. Validation of the 8th edition of the AJCC TNM staging system for gastric cancer using the national cancer database. Ann Surg Oncol 2017;24(12):3683–91.

[3] González CA, Sala N, Rokkas T. Gastric cancer: epidemiologic aspects Sep [cited 2017 Oct 13]. Available from:. Helicobacter [Internet] 2013;18(Suppl 1):34–8http://doi.wiley.com/10.1111/hel.12082.

[4] Tan P, Yeoh K-G. Genetics and molecular pathogenesis of gastric adenocarcinoma 1153–1162.e3 Oct [cited 2017 Oct 13]. Available from:. Gastroenterology [Internet] 2015;149(5)http://www.ncbi.nlm.nih.gov/pubmed/26073375.

[5] Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics [cited 2017 Oct 13]. Available from:. CA Cancer J Clin [Internet] 2001;51(1):15–36http://www.ncbi.nlm.nih.gov/pubmed/11577478.

[6] Mok YJ, Koo BW, Whang CW, Kim SM, Maruyama K, Sasako M, et al. Cancer of the stomach: a review of two hospitals in Korea and Japan. World J Surgery 1993;17:777–82http://www.ncbi.nlm.nih.gov/pubmed/8109117.

[7] Lin SJ, Gagnon-Bartsch JA, Tan IB, Earle S, Ruff L, Pettinger K, et al. Signatures of tumour immunity distinguish Asian and non-Asian gastric adenocarcinomas Nov [cited 2017 Oct 13]. Available from:. Gut [Internet] 2015;64(11):1721–31http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2014-308252.

[8] Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification [cited 2017 Oct 13]. Available from:. Acta Pathol Microbiol Scand [Internet] 1965;64:31–49http://www.ncbi.nlm.nih.gov/pubmed/14320675.

[9] Fléjou J-F. Classification OMS 2010 des tumeurs digestives: la quatrième édition [cited 2017 Oct 13]. Available from:. Ann Pathol [Internet] 2011;31(5):S27–31http://www.ncbi.nlm.nih.gov/pubmed/22054452.

[10] Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma Available from Nature [Internet] 2014;513(7517):202–9http://www.nature.com/doifinder/10.1038/nature13480.

[11] Lordick F, Janjigian YY. Clinical impact of tumour biology in the management of gastroesophageal cancer [cited 2017 Oct 13]. Available from:. Nat Rev Clin Oncol [Internet] 2016;13(6):348–60http://www.nature.com/doifinder/10.1038/nrclinonc.2016.15.

[12] Bang Y-J, Van Cutsem E, Feyereislova A, Chung HC, Shen L, Sawaki A, et al. 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 Aug 28 [cited 2017 Oct 13]. Available from:. Lancet (London, England) [Internet] 2010;376(9742):687–97http://linkinghub.elsevier.com/retrieve/pii/S014067361061121X.

[13] Fuchs CS, Tomasek J, Yong CJ, Dumitru F, Passalacqua R, Goswami C, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD): an international, randomised, multicentre, placebo-controlled, phase 3 trial [cited 2017 Oct 13]. Available from:. Lancet (London, England) [Internet] 2014;383(9911):31–9http://linkinghub.elsevier.com/retrieve/pii/S0140673613617195.

[14] Wilke H, Muro K, Van Cutsem E, Oh S-C, Bodoky G, Shimada Y, et al. 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 [cited 2017 Oct 13]. Available from:. Lancet Oncol [Internet] 2014;15(11):1224–35http://linkinghub.elsevier.com/retrieve/pii/S1470204514704206.

[15] Janjigian YY, Ott PA, Calvo E, Kim JW, Ascierto PA, Sharma P, et al. Nivolumab ± ipilimumab in pts with advanced (adv)/metastatic chemotherapy-refractory (CTx-R) gastric (G), esophageal (E), or gastroesophageal junction (GEJ) cancer: CheckMate 032 study Available from: J Clin Oncol [Internet] 2017;35(15_suppl):4014http://ascopubs.org/doi/abs/10.1200/JCO.2017.35.15_suppl.4014.

[16] Muro K, Chung HC, Shankaran V, Geva R, Catenacci D, Gupta S, et al. Pembrolizumab for patients with PD-L1-positive advanced gastric cancer (KEYNOTE-012): a multicentre, open-label, phase 1b trial [cited 2017 Oct 13]. Available from:. Lancet Oncol [Internet] 2016;17(6):717–26http://linkinghub.elsevier.com/retrieve/pii/S1470204516001753.

[17] Tan IB, Ivanova T, Lim KH, Ong CW, Deng N, Lee J, et al. Intrinsic subtypes of gastric cancer, based on gene expression pattern, predict survival and respond differently to chemotherapy 476–85, 485-11. [cited 2017 Oct 13]. Available from:.

Gastroenterology [Internet] 2011;141(2)http://linkinghub.elsevier.com/retrieve/pii/S001650851100597X.
[18] Lei Z, Tan IB, Das K, Deng N, Zouridis H, Pattison S, et al. Identification of molecular subtypes of gastric cancer with different responses to PI3-kinase inhibitors and 5-fluorouracil [cited 2017 Oct 13]. Available from:. Gastroenterology [Internet] 2013;145(3):554–65http://linkinghub.elsevier.com/retrieve/pii/S0016508513007221.
[19] Cristescu R, Lee J, Nebozhyn M, Kim K-M, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes [cited 2017 Oct 13]. Available from:. Nat Med [Internet] 2015;21(5):449–56http://www.nature.com/doifinder/10.1038/nm.3850.
[20] Bass AJ, Thorsson V, Shmulevich I, Reynolds SM, Miller M, Bernard B, et al. Comprehensive molecular characterization of gastric adenocarcinoma [cited 2017 Oct 13]. Available from:. Nature [Internet] 2014;513(7517):202–9http://www.ncbi.nlm.nih.gov/pubmed/25079317.
[21] Weis VG, Goldenring JR. Current understanding of SPEM and its standing in the preneoplastic process [cited 2017 Nov 25]. Available from:. Gastric Cancer [Internet] 2009;12(4):189–97http://link.springer.com/10.1007/s10120-009-0527-6.
[22] Schmidt PH, Lee JR, Joshi V, Playford RJ, Poulsom R, Wright NA, et al. Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma [cited 2017 Oct 13]. Available from:. Lab Invest [Internet] 1999;79(6):639–46http://www.ncbi.nlm.nih.gov/pubmed/10378506.
[23] Camargo MC, Kim W-H, Chiaravalli AM, Kim K-M, Corvalan AH, Matsuo K, et al. Improved survival of gastric cancer with tumour Epstein-Barr virus positivity: an international pooled analysis [cited 2017 Oct 21]. Available from:. Gut [Internet] 2014;63(2):236–43http://gut.bmj.com/lookup/doi/10.1136/gutjnl-2013-304531.
[24] 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 [cited 2017 Oct 21]. Available from:. Mol Clin Oncol [Internet] 2015;3(3):699–705https://www.spandidos-publications.com/.
[25] Li X, Wu WKK, Xing R, Wong SH, Liu Y, Fang X, et al. Distinct subtypes of gastric cancer defined by molecular characterization include novel mutational signatures with prognostic capability [cited 2017 Oct 21]. Available from:. Cancer Res [Internet] 2016;76(7):1724–32http://www.ncbi.nlm.nih.gov/pubmed/26857262.
[26] Kwon CH, Kim YK, Lee S, Kim A, Park HJ, Choi Y, et al. Gastric poorly cohesive carcinoma: a correlative study of mutational signatures and prognostic significance based on histopathological subtypes. Histopathology 2018;72(4):556–68.
[27] Cristescu R, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, et al. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes [cited 2017 Nov 18]. Available from:. Nat Med [Internet] 2015;21(5):449–56http://www.nature.com/doifinder/10.1038/nm.3850.
[28] Sohn BH, Hwang JE, Jang HJ, Lee HS, Oh SC, Shim JJ, et al. Clinical significance of four molecular subtypes of gastric cancer identified by The Cancer Genome Atlas project [cited 2017 Oct 21]. Available from:. Clin Cancer Res [Internet] 2017;23(15):4441–9http://www.ncbi.nlm.nih.gov/pubmed/28747339.
[29] Smyth EC, Wotherspoon A, Peckitt C, Gonzalez D, Hulkki-Wilson S, Eltahir Z, et al. Mismatch repair deficiency, microsatellite instability, and survival: an exploratory analysis of the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial [cited 2017 Dec 28]. Available from:. JAMA Oncol [Internet] 2017;3(9):1197–203http://oncology.jamanetwork.com/article.aspx?doi=10.1001/jamaoncol.2016.6762.
[30] Choi YY, Kim H, Shin S-J, Kim HY, Lee J, Yang H-K, et al. Microsatellite instability and programmed cell death-ligand 1 expression in stage II/III gastric cancer [cited 2019 Mar 14]. Available from. Ann Surg [Internet] 2018;1http://www.ncbi.nlm.nih.gov/pubmed/29727332.
[31] Kim ST, Cristescu R, Bass AJ, Kim K-M, Odegaard JI, Kim K, et al. Comprehensive molecular characterization of clinical responses to PD-1 inhibition in metastatic gastric cancer. [cited 2019 Mar 21]; Available from: https://doi.org/10.1038/s41591-018-0101-z.
[32] Setia N, Agoston AT, Han HS, Mullen JT, Duda DG, Clark JW, et al. A protein and mRNA expression-based classification of gastric cancer [cited 2017 Oct 13]. Available from:. Mod Pathol [Internet] 2016;29(7):772–84http://www.nature.com/doifinder/10.1038/modpathol.2016.55.
[33] Ahn S, Lee S-J, Kim Y, Kim A, Shin N, Choi KU, et al. High-throughput protein and mRNA expression-based classification of gastric cancers can identify clinically distinct subtypes, concordant with recent molecular classifications [cited 2017 Oct 13]. Available from:. Am J Surg Pathol [Internet] 2017;41(1):106–15http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00000478-201701000-00013.
[34] Gulmann C, Butler D, Kay E, Grace A, Leader M. Biopsy of a biopsy: validation of immunoprofiling in gastric cancer biopsy tissue microarrays. Histopathology 2003;42(1):70–6.
[35] Kim MA, Lee H-J, Yang H-K, Bang Y-J, Kim WH, Lee KMA, et al. Heterogeneous amplification of ERBB2 in primary lesions is responsible for the discordant ERBB2 status of primary and metastatic lesions in gastric carcinoma [cited 2018 Mar 4]. Available from:. Histopathology [Internet] 2011;59:822–31https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3267049/pdf/his0059-0822.pdf.
[36] Pectasides E, Stachler MD, Derks S, Liu Y, Maron S, Islam M, et al. Genomic heterogeneity as a barrier to precision medicine in gastroesophageal adenocarcinoma. Cancer Discov 2018;8(1):37–48.
[37] Corso G, Carvalho J, Marrelli D, Vindigni C, Carvalho B, Seruca R, et al. Somatic mutations and deletions of the e-cadherin gene predict poor survival of patients with gastric cancer [cited 2017 Dec 31]. Available from:. J Clin Oncol [Internet] 2013;31(7):868–75http://ascopubs.org.sire.ub.edu/doi/pdfdirect/10.1200/JCO.2012.44.4612.
[38] Ichikawa H, Nagahashi M, Shimada Y, Hanyu T, Ishikawa T, Kameyama H, et al. Actionable gene-based classification toward precision medicine in gastric cancer. Genome Med 2017;9(1).
[39] Kim HS, Lee H, Shin S-J, Beom S-H, Jung M, Bae S, et al. Complementary utility of targeted next-generation sequencing and immunohistochemistry panels as a screening platform to select targeted therapy for advanced gastric cancer Available from Oncotarget [Internet] 2017;8(24):38389–98http://www.ncbi.nlm.nih.gov/pubmed/28418920%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5503540.