Review

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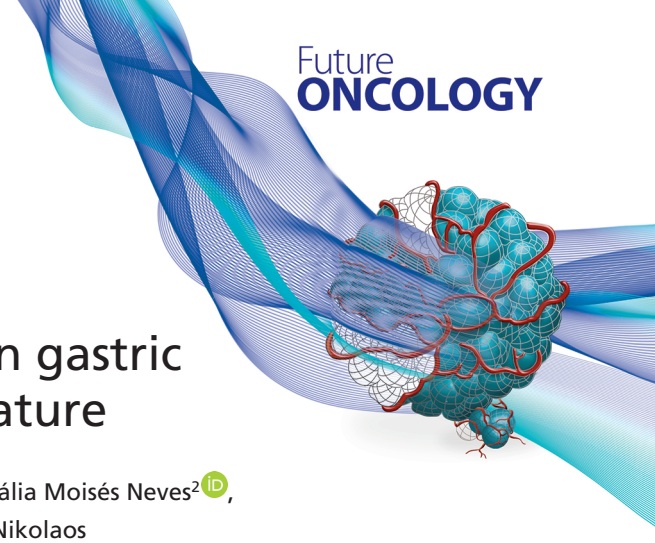

Current and potential biomarkers in gastric cancer: a critical review of the literature

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Gastric cancer is the fourth most common type of cancer worldwide and the second most lethal. Gastric cancer biomarkers can be used for diagnosis, prediction of sensitivity to treatment, and prognosis. The following search terms were applied to PubMed as of December 2020: 'gastric cancer classification', 'gastric cancer epidemiology', 'cancer metastasis' and 'gastric cancer biomarker'. Only experimental studies were reported in the 'biomarkers' section. Some biomarkers can serve as therapeutic targets for antitumoral drugs. The genes analyzed include E-cadherin, *RPRM*, *XAF1*, *MINT25*, *TFF1*, *p16* and *p53*. The miRNAs analyzed include miR-18a, miR185-5p, miR-125b and miR-21. Some molecules were associated with metastasis of gastric cancer, specifically those involved with EMT process and tissue degradation.

Lay abstract: Gastric cancer is the fourth most common type of cancer worldwide and the second most lethal. Gastric cancer biomarkers are molecules that have different expressions in tumor cells than in normal body cells, and can be used for diagnosis, prediction of sensitivity to treatment, and prognosis. Biomarkers in gastric cancer can include genes that suppress tumor progression, genes that increase tumor progression by binding to growth molecules, molecules related to the body's immune response to the tumor, and non-coding RNA molecules (RNA molecules that do not produce proteins but regulate the cell's genetic material). Some biomarkers can serve as therapeutic targets for anti-tumoral drugs.

First draft submitted: 21 January 2021; Accepted for publication: 9 June 2021; Published online: 22 July 2021

Keywords: biomarkers • diagnosis • gastric cancer • literature review • prognosis • sensitivity • treatment

Gastric cancer (GC), a malignancy which most commonly presents as adenocarcinoma, can be classified into four main molecular subtypes: GC with associated chromosomal instability, genetically stable tumors, Epstein–Barr virus (EBV)-positive tumors and GC with associated microsatellite instability (MSI) [1]. The most frequent anatomical location of each type of GC (cardia, body, antrum) and some characteristics associated with each type are shown in Figure 1. Gastric carcinogenesis is determined by the interaction of numerous genetic, epigenetic and environmental factors. The disease occurs mostly sporadically, due to the occurrence of somatic genetic alterations, with less than 15% having a familial component and less than 3% of GCs being related to hereditary syndromes such as diffuse hereditary cancer (germline mutation of *CDH1*), Peutz–Jeghers syndrome, Li–Fraumeni syndrome, familial adenomatous polyposis and hereditary non-polyposis colorectal cancer [2].

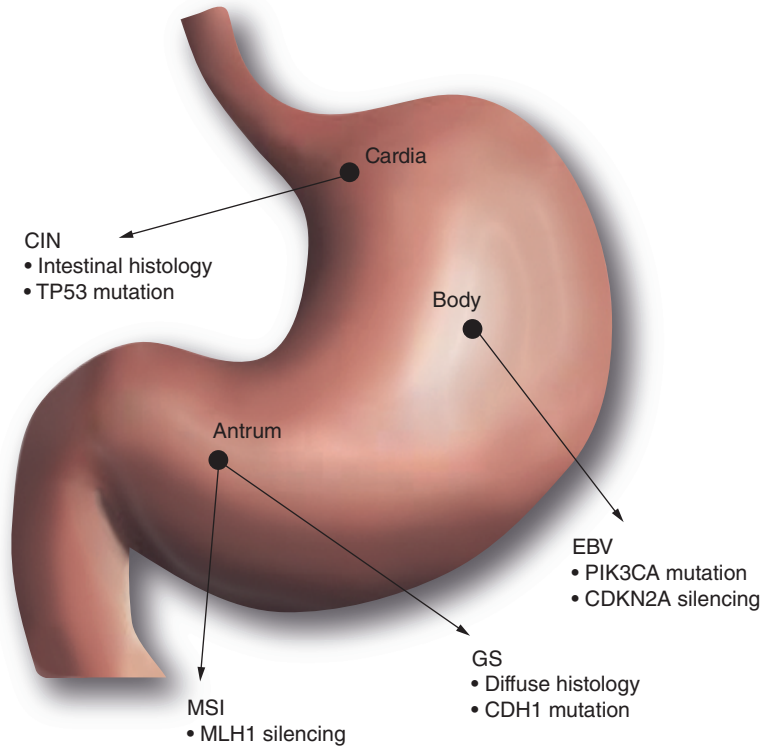

Figure 1. The most frequent anatomical location of each molecular type of gastric cancer and the characteristics associated with each type.
CIN: Gastric cancer with chromosomal instability; EBV: Epstein–Barr virus-positive gastric cancer; GS: Genetically stable gastric cancer; MSI: Gastric cancer with associated microsatellite instability.

In terms of epidemiology, it is worth noting that over 50% of all GC cases occur in East Asia, and Asian heritage has long been described as a GC risk factor [3]. Other important risk factors associated with all types of GC are advanced age (the median age for diagnosis is 70 years), male sex (the incidence of GC in males can be up to five-times the incidence in females), tobacco usage (this increases the risk of GC by 60% in males and 20% in females). Infection by *Helicobacter pylori* has been described as a risk factor specifically for non-cardia GC, increasing the risk of this type of malignancy by up to 80%. Other risk factors that are specific to non-cardia GC include low intake of fruits and vegetables and heavy consumption of salty and smoked foods [4]. Infection by EBV also increases the risk of GC, and studies have shown that around 10% of all gastric carcinomas are EBV positive [5,6].

Despite the decreasing incidence of GC over the past 70 years, this disease remains the fourth most common malignancy worldwide and the second most lethal, causing an estimated 650,000 deaths per year [7]. Studies also show that GC entails a high burden on patients, accounting for 20% of all disability-adjusted life years lost worldwide [8]. Given these conditions, the catalog of biomarkers associated with GCs is of utmost importance. These markers serve as current and potential targets for more effective diagnosis, more accurate prognosis and less debilitating cancer therapies.

Materials & methods

The information presented in this review was selected from articles present in the NIH PubMed database as of March 2021. The authors decided to use PubMed's 'Best Match' algorithm to order the results of the search. The aforementioned algorithm gives higher weight to more relevant and recently published articles. The authors also considered PubMed's list of 'similar articles' for inclusion. The articles selected through PubMed were published from 1999 to 2020. They were all written in English but there was no restriction made for the country of origin. For the introductory portion of the article, two different terms were inserted in turn in the PubMed search engine: 'gastric cancer classification' and 'gastric cancer epidemiology'. For the 'Biology of tumorigenesis' section, the

search term was 'cancer metastasis'. For the 'Gastric cancer biomarkers' section, the search term was 'gastric cancer biomarkers', and the authors decided to select only experimental studies; review articles were disregarded.

Besides PubMed, other resources were used in the making of this article. In the 'Biology of tumorigenesis' section, some of the information was gathered from a prominent genetics textbook [7]. The Discussion section mentions ten current studies on the topic of GC, which were found through the NIH's clinicaltrials.gov database, again using the keywords 'gastric cancer biomarkers' in the search engine. No specification was made for the country of origin or status of the study.

The information gathered through all the cited sources that was found relevant to the topic at hand was analyzed and synthesized by the authors, who also analyzed the statistical relevance and risk of bias reported in the studies and their credibility. The overall quality of the studies was analyzed by the source of the publication (only articles selected from journals with an impact factor of 3.000 or higher were considered) and date of publication (preference was given to recently published works, especially in the sections of the article regarding upcoming biomarkers such as noncoding RNAs and immune-related biomarkers).

Results & discussion

Biology of tumorigenesis

Cancer is considered a multifactorial disease that develops due to the accumulation of both genetic and epigenetic changes, which contribute to an unruly multiplication of altered cells. The cells of malignant tumors have heterogeneous characteristics due to the presence of genetic and phenotypic amplifiers. But cancer cells as a whole share malignancy patterns, which are: high proliferative power, angiogenic capacity, metastatic potential, evasion of the immune system, chemoresistance and the presence of epithelial–mesenchymal transition (EMT). Ten distinct biological characteristics, called hallmarks of cancer, are acquired in the course of tumor development and can significantly determine the process of carcinogenesis. These hallmarks are: proliferative signaling capacity, inhibition of growth-suppressive signals, evasion of immune destruction, mechanisms of resistance to apoptosis, tumor-promoting inflammation, replicative immortality, genomic instability, induction of angiogenesis, ability to metastasize and deregulation of cellular metabolism. The acquisition of these functions in the course of tumorigenesis is made possible with the appearance of genomic instability in tumor cells, which generates a propensity for mutations and chromosomal rearrangements. The inflammatory process present in the neoplastic environment also enhances the propensity for mutations [9].

The tumorigenesis of several cancers can be regulated by signaling molecules, such as TGF and FGF, which activate signaling pathways like Wnt that will ultimately result in the expression of characteristics of cell malignancy. This signaling can control both the differentiation and the development of embryonic neural precursor cells, as well as cancer cells, which demonstrate similarity between their regulatory mechanisms. This similarity suggests that tumor cells may share characteristics of embryonic neural cells [10]. Tumorigenesis can also be guided by enzymes that change the cell's chromatin in a process called neoplastic reprogramming.

The genes involved in carcinogenesis are divided into two main classes: proto-oncogenes and tumor suppressor genes. Proto-oncogenes stimulate cell proliferation (through reduced apoptosis and increased mitotic activity) and stimulate the invasion of adjacent tissues by these cells. Tumor suppressor genes inhibit cell proliferation (through increased apoptosis and reduced mitotic activity) and inhibit the invasion of adjacent tissues by these cells. Gene expression in carcinogenesis is modulated by genetic and epigenetic alterations. The best-known type of epigenetic modification is DNA methylation, which consists of the addition of a methyl group in a gene's CpG islands located inside the promoter portion of the gene. During carcinogenesis, hypermethylation silences tumor suppressor genes, while hypomethylation activates oncogenes, as shown in Figure 2 [11].

The expression of proto-oncogenes and tumor suppressor genes is also modulated by noncoding RNAs, which are divided into lncRNAs and miRNAs. The main difference between these two classes is the length of the molecule: lncRNAs have a length of over 200 nucleotides, while miRNAs consist of up to 25 nucleotides [12]. Noncoding RNAs can be methylated, having their expression increased by hypomethylation or decreased by hypermethylation, which therefore alters the cell's gene expression.

With altered gene expression, cancer cells acquire, among other characteristics, independence from growth factors, immune resistance, increased proliferation, decreased apoptosis and the ability to form metastases.

The increased proliferation of cancer cells is mediated by a higher expression of certain oncogenes that interfere in the cell cycle, such as *HER2* and *MYC*. The *HER2* gene encodes an endothelial growth factor receptor with intrinsic tyrosine kinase activity. When growth factors bind to these receptors, they dimerize and their catalytic

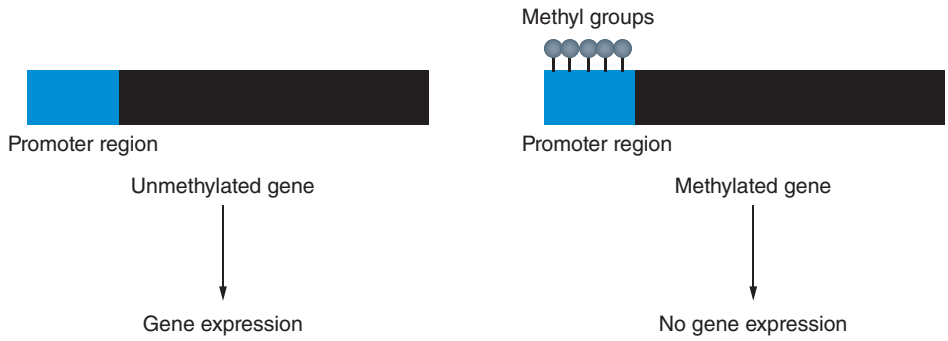

Figure 2. Epigenetic alteration consisting of the methylation of a gene's promoter region, which inhibits gene expression.

activity is activated, leading to the activation of intracellular signaling pathways that stimulate cell division (MAPK pathway) and pathways that inhibit apoptosis (PI3K). Activation of the *HER2* proto-oncogene occurs through gene amplification, leading to an overexpression of receptors on the tumor cell surface. MYC is a transcription factor; when activated, it enters the cell nucleus and stimulates the expression of several genes related to mitochondrial metabolism, protein synthesis and DNA replication. MYC can be activated by several signaling pathways, among them MAPK, which is initiated by activation of HER2 receptors. This shows that the mechanisms of oncogenes are interconnected [11].

In terms of apoptosis, cancer cells can escape and resist cell death through many mechanisms; one of the main pathways involves the silencing of the tumor suppressor gene *p53*. In normal situations, the protein BCL2 inhibits the caspase proteins involved in cell death. When there is DNA damage, a signaling pathway leads to the production of the p53 protein, which stimulates the expression of molecules that inhibit BCL2, activating apoptosis. Thus the loss of both alleles of *p53* leaves the tumor resistant to apoptosis, because BCL2 will not be inhibited and therefore continues blocking the caspase chain reaction [11].

Cancer cells obtain the capacity to invade adjacent tissues mainly through EMT and expression of matrix metalloproteinases (MMPs). In the EMT process, epithelial cells lose the junctions that bind them to adjacent cells and also undergo morphological changes in their cytoskeleton, turning into nonpolarized mesenchymal cells that have high mobility and are therefore capable of invading surrounding tissues. EMT is induced by a variety of growth factors, such as TGF-β, HGF, EGF and FGF. Important pathways in the EMT process include MAPK and Jagged1/Notch [13]. The expression of MMPs is also important in metastasis, because these proteins are enzymes capable of degrading the basal membrane and the extracellular matrix, allowing the cancer cells to break down physical barriers and penetrate adjacent tissues. MMPs are secreted as zymogens and activated through plasmin, urokinase plasminogen activator and other proteases [14]. Another important group of molecules in determining metastasis consists of the VEGF family. These molecules bind to tyrosine kinase receptors to determine angiogenesis, which gives the growing tumor a supply of necessary nutrients [15].

GC biomarkers

Tumor antigens

Currently, some of the most commonly used biomarkers for GC are the same ones used for other types of malignancies in the digestive system and elsewhere in the body. Among these, we find carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA 19-9), AFP and carbohydrate antigen 125 (CA-125). Among these biomarkers, CEA can be used somehow to predict prognosis. CEA-positive GC patients have an overall survival rate that is decreased by around 25% compared with CEA-negative patients [7]. These biomarkers can also be used for diagnosis, but they are not ideal because they are relatively rarely found in early GC. The highest positive rate found with these biomarkers was 10.4% when a combination of all four was tested in GC patients for diagnosis [16]. CA 72-4 is another serum cancer antigen that has been tested in a Taiwanese study as a potential biomarker for GC screening [16]. Although specificity was high, sensitivity and positive predictive value was low. Elevation of CA 72-4 was also associated with other conditions, such as gastric ulcer, polyps and gastritis [17]. Pepsinogen, the proenzyme of pepsin, is released by the chief cells of gastric mucosa and is an important component of physiological stomach

function. Pepsinogen is divided into two subtypes, PGI and PGII. In the setting of atrophic gastritis, *H. pylori* infection or gastric carcinoma, PGI expression and PGI/II ratio are reduced [18]. The value of pepsinogen as a screening biomarker has been further tested in a Korean case–control study of 398 patients, including 87 with gastric neoplasm. The PGI/II ratio was highly sensitive (97.7%) in the detection of gastric neoplasms at a cut-off of 4.5, albeit at the cost of low specificity (57.6%) [19] A novel biomarker that is expressed in many different neoplasms is progastrin, the precursor molecule of gastrin. Progastrin can be measured at increased concentration in the serum of patients with different solid tumors, including GC. Changes in progastrin serum concentration have also been related to anticancer treatment efficacy. Progastrin can function as a predictive biomarker as well as a potential drug target, due to its role in the activation of the Wnt/β-catenin pathway. Most of the biomarkers mentioned above lack specificity for GC, as they are expressed in a variety of solid tumors, including liver and colorectal cancer [20]. Regardless of these shortcomings, it is clear that the aforementioned biomarkers are important in clinical practice. However, the need for biomarkers that will be more specific and sensitive to GC (and to different stages of previously mentioned groups of tumors) can improve diagnosis, treatment and outcomes.

Tumor-related genes

Studies have found consistent hypermethylation of around 70 tumor suppressor genes in cases of GC. Some tumor suppressor genes can be hypermethylated under the influence of *H. pylori* (E-cadherin/*CDH1*) and EBV (E-cadherin/*CDH1*, *p14*, *p15*, *p16*) [21,22]. Therefore these are biomarkers associated with the carcinogenesis of this malignancy and can potentially be used for diagnosis. Other hypermethylated genes in GC were found to be detectable in patients' serum and can therefore be used as biomarkers for the diagnosis of GC. Some of the most promising genes for this purpose are *RPRM* (sensitivity of 95.3% and specificity of 90.3%), *XAF1* (methylated fragments of this gene were not found in the controls), *CYP26B1* and *KCNA4*. A combination of these last two genes provides a diagnostic biomarker with a sensitivity of 91.3% and a specificity of 92.1% [23–25]. In gastric washes, *MINT25* is the most commonly found hypermethylated gene and is a highly sensitive (90%) and specific (96%) biomarker for diagnosis through this procedure [26]. Other biomarkers of GC can predict the patients' outcomes after treatment. Some genes, when found to be hypermethylated, predict a poor outcome; E-cadherin, an important gene in maintaining cell adhesion, is one of them. Patients with hypermethylated E-cadherin can have their 5-year survival rate decreased by 32% [27]. *CACNA2D3*, a gene that produces a subunit in voltage-dependent calcium channels, when found hypermethylated, decreased survival rate by about 32% [28]. Amplification of *HER2* in GC patients has been found to decrease the median survival rate by one-half [29]. Figure 3 showcases PI3K, one of the signaling pathways activated by HER2, which stimulates cell survival by blocking apoptosis [30]. Gastric tumors with *HER2* amplification confirmed by immunohistochemistry (IHC), *in situ* hybridization or next-generation sequencing can be targeted with HER2-binding antibodies. Trastuzumab, a monoclonal antibody specific for the extracellular domain of HER2 receptor, has received US FDA approval for the treatment of patients with metastatic or locally advanced GC with *HER2* amplification in the first-line setting, in combination with fluorouracil-based chemotherapy, based on the results of the ToGA Phase III trial [27,28]. Furthermore, there are data to suggest the efficacy of anti-HER2 treatment in early GC, with the use of trastuzumab and pertuzumab, another HER2-binding monoclonal antibody, which prevents heterodimerization with the HER3 receptor [30]. Patients with resectable GC in the PETRARCA study who received neoadjuvant chemotherapy with the addition of trastuzumab and pertuzumab achieved higher pathological complete response rates and longer disease-free survival [29]. Lately, the antibody–drug conjugate fam-trastuzumab deruxtecan received FDA approval for the treatment of metastatic or locally advanced GC that has progressed on at least two lines of previous therapy, including trastuzumab-based treatment, based on the results of the Phase II DESTINY-Gastric01 trial, which showed a statistically significant improvement in overall survival as well as a high rate of objective response (51%) and disease control (86%) [31]. The overexpression of *FGFR2* in GC is associated with poor survival rates ($p < 0.0001$) [31]. The recent Phase II FIGHT study showed an improvement in progression-free survival and a statistically significant improvement in overall survival in patients with gastric and gastroesophageal junction (GEJ) cancers and *FGFR2b* overexpression by IHC or by ctDNA, that received the novel anti-FGFR2b monoclonal antibody bemarituzumab in combination with FOLFOX, in the first-line setting [32]. The *PD-L1* gene produces a receptor of the same name whose function is to limit the activation and proliferation of T cells, helping the cancer cells escape the body's immune response. PD-L1 can be useful for GC diagnosis; a study found the circulating levels of this gene highly upregulated in 58.8% of patients [32]. Another study shows this gene is also promising for prognosis: high *PD-L1* expression was found to be linked with a 36% lower 5-year post-gastrectomy survival rate [33]. Targeting

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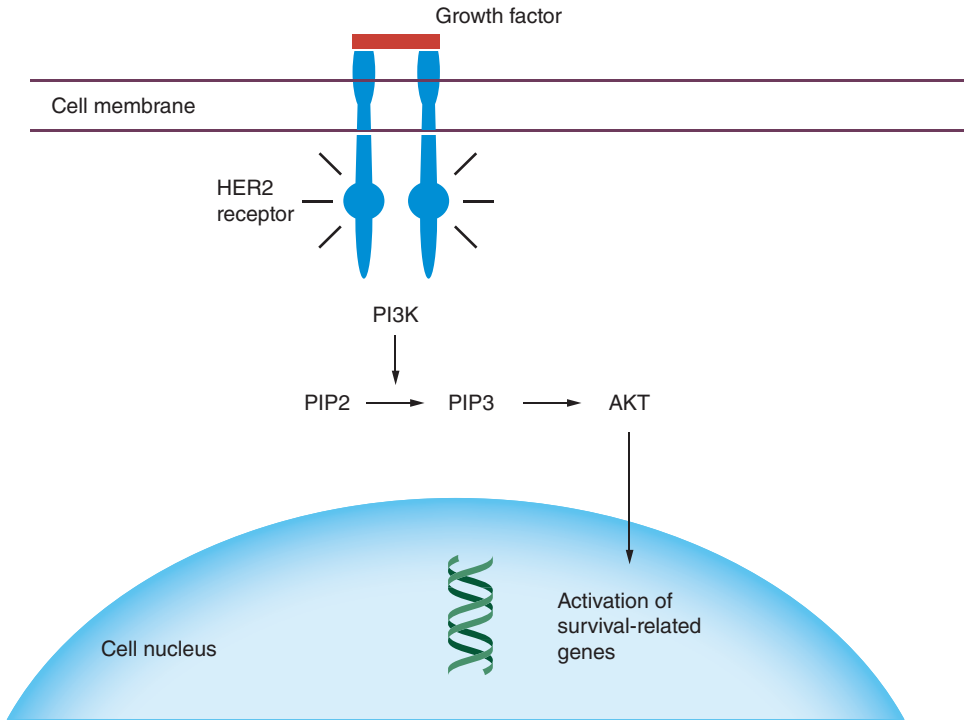

Figure 3. Activation of HER2 receptor by growth factors, which activates the PI3K pathway, concluding in activation of genes that inhibit apoptosis/promote cell survival.

the PD-1 and PD-L1 molecules with monoclonal antibodies induces an immune response against tumor cells and has been proved effective in the treatment of various cancers including lung, melanoma and renal cell carcinoma. In patients with GC, pembrolizumab, an anti PD-1 antibody, has been approved as monotherapy in patients with a Combined Positive Score (CPS) >1% in the third or subsequent line of treatment based on the results of KEYNOTE-059 study [34]. Furthermore, the newer Phase III studies Keynote-062 and CheckMate 649 suggest a role for PD-1 inhibitors pembrolizumab and nivolumab in the first-line setting in patients with positive CPS [31]. Moreover, preliminary results from the Phase III CheckMate 577 study in patients with early-stage esophageal and GEJ tumors that underwent surgical resection after neoadjuvant chemoradiation showed a doubling in disease-free survival (22.4 months) with the administration of adjuvant nivolumab for up to 1 year or disease relapse [35]. Finally, data from the early Phase Ib/II PANTHERA trial suggest a possible benefit with the combination of trastuzumab and pembrolizumab with chemotherapy as first-line treatment in patients with HER2-positive advanced gastric or GEJ cancers. Overall response rate reached 76.7% and disease control rate 97.7% of 43 treated patients, with a median progression-free survival of 8.6 months and an overall survival of 18.4 months. Over one-half (57.1%) of patients had a CPS >1% [36]. Other biomarkers can be used to predict a patient's resistance or sensitivity to GC treatment, allowing for more personalized and effective therapy. A hypermethylated *p16* gene increases sensitivity to 5-fluorouracil, a commonly used drug [35]. High expression of *p53* is associated with resistance to cisplatin-based therapies in GC [36]. Another important gene in the path of tumor progression in GC is c-MET proto-oncogene, a member of the RTK family, which encodes a receptor for HGF. Activation of the receptor leads to activation of multiple signaling pathways, such as PI3K/AKT and MAPK, inducing tumor survival and progression. MET protein expression on IHC has been associated with prevalence of intestinal type in GC, as well as with advanced tumor stage, lymph node metastases and poor survival [36]. Multiple MET inhibitors have been tested in the preclinical setting in GC cell lines and xenografts. Several MET inhibitors have also been tested in clinical trials, such as onartuzumab, which failed to improve outcomes in two Phase II trials, whereas new agents such as capmatinib, which has shown activity in *MET* exon 14 skipping mutation in lung cancer, are now being tested in Phase I trials for safety and efficacy in MET-positive GC [35,36].

Immune-related biomarkers

Tumor biology and behavior do not depend solely upon intrinsic factors but are also affected by the tumor microenvironment. Cancer cells have an interactive relationship with their surroundings, receiving signals from stromal cells, as well as creating a favorable milieu for progression and metastasis. As already mentioned, an important hallmark of cancer is immune evasion, which is achieved through changes in tumor recognition molecules, as well as through changes in tumor-infiltrating immune cells. A particular set of cells that facilitate tumor evasion are Treg cells, a subset of $CD4^+$ $CD25^+$ T cells, which physiologically suppress the immune response to avoid extended damage to normal tissues. In cancer tissue stroma, Tregs are elevated, as identified by the FOXP3 protein, halting antitumor immune response and facilitating tumor progression. As a result, high levels of tumor-infiltrating $CD4^+$ $FOXP3^+$ Tregs in patients with GC and other solid tumors have been associated with advanced stages of disease and worse outcomes [37]. The type of circulating white blood cells can also predict prognosis of patients with malignancies. Miyamoto *et al.* investigated the prognostic value of neutrophil-to-lymphocyte ratio (NLR) in patients with resectable GC in 154 patients undergoing surgical resection. Median overall survival and median disease-free survival differed significantly among groups, with an increased risk of death or early relapse in the subgroup of patients with high NLR. Perioperative complications were also higher in the high-NLR subgroup [37]. Accordingly, Murakami *et al.* explored the prognostic role of NLR in a study of 92 patients with unresectable GC. Patients with advanced disease had an increased NLR compared with those with recurrent disease and demonstrated lower overall survival [35–37]. In a systematic review by Zhou *et al.*, both high NLR and $FOXP3^+$ Tregs were associated with worse overall survival in patients with GC [37].

Noncoding RNAs

Some lncRNAs are also associated with the development of GC and can be used as biomarkers. *CASC15* is an example of one lncRNA correlated with tumorigenesis. This RNA causes hypermethylation of the *CDKN1A* gene, which leads to increased cell proliferation and migration [37]. Other lncRNAs can be used for GC diagnosis: *H19* (sensitivity of 82.9% and specificity of 72.9%) and *PCGEM1* (sensitivity of 72.9% and specificity of 88.9%) are some of the most promising [38,39]. lncRNAs with prognostic value include the aforementioned *CASC15*. Another study tested a combination of 24 different lncRNAs and found that patients with high expression of these molecules had disease-free survival rates decreased by around 60% compared with patients who had low expression of these RNAs [40]. lncRNAs are also useful to predict drug sensitivity in GC patients. High expression of *CASC9* was found to decrease resistance to paclitaxel and adriamycin, while high expression of *MRUL* was found to decrease resistance to adriamycin and vincristine [41,42]. High expression of *MALAT1* induces resistance to cisplatin (CDPP) and vincristine, while high expression of *ANRIL* induces resistance to CDDP and 5-fluorouracil [43,44].

Circular RNAs

Circular RNAs consist of closed RNA molecules formed by an alternative splicing method in which covalent bonds in the molecule form a looped structure. Ever since their discovery in the 1970s, new studies have emerged showing the role of circRNAs in cancer progression. The high expression of circRNAs in cancer cells, combined with these molecules' high stability when compared with the linear RNA, makes them a promising target for biomarkers. Recent studies have found an aberrant expression of 214 different circRNAs in GC and a decreased expression of 253 other circRNA molecules in this malignancy [45]. Some of the circRNAs with the most potential as GC diagnostic biomarkers include circPSMC3, which showed a sensitivity of 85.85% and a specificity of 95.24% [46]. This same molecule also presents a prognostic value, since its low expression by GC tissues has been associated with protection against lymph node metastasis [47]. Another potential diagnostic and prognostic circRNA is hsa_circ_0001649, which showed a sensitivity of 71.1% and a specificity of 81.6%; lower expression of this molecule was associated with low differentiation and therefore higher aggressiveness of GC cells [46]. Furthermore, some circRNAs can also act as biomarkers of drug resistance. Molecules such as hsa_circ_0081143 and circAKT3 have been found to induce resistance to cisplatin-based regimens of chemotherapy in GC cells, through the upregulation of the CDK6 and PIK3 pathways, respectively [47].

MicroRNAs

MicroRNAs are also associated with the development of GC and can be used as biomarkers. High levels of miR-296-5p and miR-301a contribute to proliferation and invasion of GC cells because these miRNAs decrease the expression of the tumor suppressor genes *CDX1* and *RUNX3*, respectively [48,49]. Other altered miRNAs

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in GC include miR18a, miR10b-5p, miR132-3p, miR185-5p, miR195-5p, miR-20a3p and miR296-5p. The aforementioned molecules are overexpressed in cancerous cells and can serve as biomarkers for diagnosis because their increased levels show up in the patients' plasma. The expression of miR-18a, for example, was found to be significantly higher in GC than in normal gastric tissue ($p = 0.0286$) [50,51]. Other possible biomarkers for diagnosis include miR-133a and miR-421. These miRNAs are present in the gastric fluid of GC patients at a lower rate than in that of non-GC patients; the levels of miR-133a can be up to 57.6% lower in GC tissue than other types of gastric tissue. miR-421 has a sensitivity of 71.4% and a specificity of 71.7% for diagnosis [52,53]. miRNAs in GC are also associated with response to chemotherapy and therefore can be used as biomarkers to predict the efficacy of a certain treatment. For example, high expression of miR-125b has been linked with dismal responses to drugs such as trastuzumab ($p = 0.047$) [54]. High expression of miR-31, on the other hand, elevated patients' sensitivity to treatment with 5-fluorouracil ($p = 0.0001$) [55]. Other miRNAs can serve as valuable biomarkers for the prediction of patient outcomes in GC. High levels of miR-21 and miR-23b correlated with lower 3-year and 5-year survival rates, respectively [56,57], while low expression of miR-144 is associated with a 50% reduction in 5-year overall survival rate [58,59].

Pathways of metastasis

Metastasis is the main cause of mortality in cancer patients, accounting for over 90% of deaths [60]. GC spreads most frequently to the liver, peritoneum and lungs [60]. In GC, the main pathways that promote EMT are PI3K/AKT/mTOR and WNT/β-catenin [61]. In metastatic GC cells, there has been found to be upregulation of the following molecules that promote EMT: SRF (a transcription factor associated with inhibiting the production of E-cadherin), miR-544a (associated with inhibiting the production of E-cadherin, APC2 and AXIN2), EphA2 (a tyrosine kinase that stimulates the WNT/β-catenin pathway) and JMJD2B (a hydroxylase protein that stimulates the WNT/β-catenin pathway) [62–65]. Also contributing to metastasis in GC, MMP-7, MMP-11 and MMP-14 are commonly found to be activated or overexpressed; MMP-14, for example, has diagnostic value because it has a higher expression in GC tissue than in normal gastric tissue ($p = 0.037$) []66. This molecule also predicts poorer survival rates ($p < 0.001$) [66–68]. As previously mentioned, the VEGF family also plays a crucial role in metastasis. VEGF-C and VEGF-D have diagnostic potential because they are found to be underexpressed and overexpressed in GC patients, respectively ($p < 0.001$) [69]. VEGF molecules also predict a reduction in overall survival time ($p = 0.040$) [70]. Due to the importance of tumor angiogenesis in GC progression, antiangiogenic agents targeting VEGF have been employed in the treatment setting. Ramucirumab, a direct VEGFR2 antagonist, has been approved in combination with paclitaxel as second-line treatment after progression on a first-line platinum–fluorouracil combination, based on the results of the Phase III RAINBOW study [69,70]. The RAMSES Phase II study evaluated the role of ramucirumab in resectable GC, when administered as neoadjuvant chemotherapy in combination with FLOT [69,70]. Patients in the ramucirumab–FLOT arm achieved higher rates of R0 excision compared with the control arm; however, no difference in overall survival was noted. Another monoclonal antibody, bevacizumab, which inhibits VEGF-A and has improved outcomes in patients with colorectal cancer, failed to elicit an improvement in overall survival in GC in the Phase 3 AVAGAST trial, although it improved progression-free survival in the first-line setting in combination with platinum–fluorouracil treatment [70,71]. Another useful prognostic marker is the degree of infiltration of tumor stroma by cancer stem cells, which has been associated with unfavorable prognosis due to facilitating tumor aggressiveness and metastasis. In a meta-analysis of 26 studies including 4729 patients with GC, Lu *et al.* examined the prognostic role of cancer stem cell markers CD44 and CD133 [71,72]. High CD44 expression was associated with intestinal type and lymphatic vessel invasion, whereas CD133 overexpression was related to more advanced tumor node metastasis stage, higher depth of invasion and increased chance of vascular invasion, lymph node and distant metastasis. Both markers were associated with lower 5-year overall survival in a statistically significant manner. CLDN18.2 is a protein belonging to the protein family of claudins, which regulate the activity of tight junctions and the movement of molecules among cells. In tumor tissues, claudins are exposed to the extracellular environment, making it a potential drug target. Zolbetuximab is the first monoclonal antibody binding the epitope of the exposed CLND18.2, and has produced results suggesting clinical efficacy in the Phase IIa MONO study, where it achieved a clinical benefit rate of 23% and an overall response rate of 9% in patients with moderate to high (>50% of tumor cells) expression of CLND18.2 [72,73]. In conclusion, all of the molecules mentioned in this section promote metastasis and are therefore potential biomarkers for GC diagnosis, prognosis and treatment.

Table 1. Biomarkers that serve as targets for anti-GC drugs and the result of treatment with said medicines.

Targeted biomarker	Drug	Median overall survival time increase
EGFR family	Cetuximab	5.4–16 months
EGFR family	Panitumumab	11.3 months
HER-2	Trastuzumab	13.8 months
Matrix metalloproteinases	Marimastat	45% improved median survival time
VEGF	Bevacizumab	10.1–12.1 months

Data from [71–76].

The Cancer Genome Atlas classification

As mentioned before, analysis from the Cancer Genome Atlas project classifies GC into four different categories based on their genomic profile: EBV-positive, MSI, chromosomally unstable, genetically stable.

Tumors containing EBV account for approximately 10% of GC and are characterized by a high prevalence of DNA hypermethylation and amplification of *JAK2*, *PD-L1* and *PD-L2*. Moreover, nearly 80% have a protein-changing alteration in the *PIK3CA* gene pathway [74,75]. EBV-associated tumors are usually located in the proximal stomach and are associated with diffuse type [76]. A large meta-analysis of multiple multicenter studies concluded that EBV^+ GC has more favorable outcomes compared with EBV^- subtypes [68,69]. Furthermore, Sohn *et al.* concluded that the EBV-positive subtype has the best prognosis among all other GC subtypes [70,71]. Patients with EBV^+ showed a high response rate in a Phase II study of 61 patients with GC who received pembrolizumab as salvage treatment. All six EBV-positive patients achieved partial response [72,73].

MSI is present in around 20% of GCs [74,75]. Tumors showing microsatellite instability contain a high rate of mutations, including mutations of genes encoding targetable oncogenic signaling proteins, caused by malfunctioning of the DNA repair mechanisms. These tumors are characterized by *MLH1* hypermethylation and CIMP [72,73]. GC with MSI has a worse prognosis than EBV-positive GC, but the prognosis is better than that of the genetically stable subtype, according to Sohn's prognostic model [73,74]. Pembrolizumab, an anti-PD-1 antibody, has shown efficacy in MSI-H gastric tumors in a trial by Le *et al.* which explored the effectiveness of pembrolizumab in various high-MSI solid tumors, and has been approved as second-line treatment for patients with high-MSI or mismatch repair-deficient tumors [72,73].

Chromosomally unstable GCs are the most frequent type, accounting for around 50% of GC specimens, and they usually appear at the GEJ. These tumors display marked aneuploidy and have a considerable number of genomic amplifications of key receptor tyrosine kinases, cell cycle regulation genes and transcription factors. They are associated with intestinal histology and most carry *p53* mutations and *RTK-RAS* activation [73,75]. The prognosis is similar to that of the MSI subtype; however, chromosomally unstable GCs seem to receive the largest benefit from adjuvant chemotherapy [7,39].

The genomically stable subtype of GC lacks the molecular characteristics of the other three subtypes and has tumors enriched for the diffuse histological variant, with approximately 30% having mutations or fusions in *CDH1* and the RHOA signaling pathway. This group accounts for 20% of GCs that are characterized by a lack of high levels of aneuploidy and high metastatic potential. It carries the worst prognosis of all the subtypes and receives little benefit from adjuvant chemotherapy, according to Sohn's model [40,41]. *CDH1* germline mutations are usually associated with the hereditary diffuse gastric cancer syndrome.

While all of the aforementioned biomarkers for GC are potential therapeutic targets, some of the current targets for GC treatment are summarized in Table 1 [71–76].

Future perspective

The analysis of the biomarkers associated with GC shows a wide variety of molecules with diagnostic and prognostic value, besides being important in predicting drug sensitivity. This opens up the prospect of widening the array of biomarkers tested clinically for GC diagnosis and prognosis, though more profound research is needed to determine which combination of biomarkers gives the best sensitivity and specificity for diagnosis and is the most accurate for prognosis. The combination of biomarkers from different molecular groups could potentially increase diagnostic and prognostic precision. It is also important that the molecules tested prove to be more specific for GC tumors,

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Table 2. Ten current studies investigating gastric cancer biomarkers.

Clinical trials (search on 3 Jan 2021)				
Study title	**Status**	**Intervention**	**Location**	**Time frame**
Biomarker-Integrated Umbrella, Advanced Gastric Cancer	Recruiting	Biomarker screening (immunohistochemistry and *in situ* hybridization)	Yonsei Cancer Center (Seoul, Korea)	2016–2021 (e)
Predicting Biomarker of Gastric Cancer Chemotherapy Response	Recruiting	Chemotherapy	Kyungpook National University Medical Center (Daegu, Korea)	2015–2022 (e)
Biomarker-oriented Study of Durvalumab (MEDI4736) in Combination with Olaparib and Paclitaxel in Gastric Cancer	Recruiting	Paclitaxel, Olaparib, Durvalumab	Seoul National University Hospital (Seoul, Korea)	2018–2020 (e)
The Value of TFF3 in Diagnosis of Gastric Cancer	Recruiting	Diagnostic Test: Trefoil factor family 3 (TFF3), Pepsinogen 1 (PG1)	Melouk Ahmed Mahmoud (Assiut, Egypt)	2019–2022 (e)
Potential Clinical Utilities of Circulating Tumor DNA in Gastric Cancer	Recruiting	Combination Product: AVENIO ctDNA surveillance kit	ZhongShan Hospital FuDan University Shanghai (Shanghai, China)	2018–2021 (e)
Prediction of the Efficacy of ctDNA in Immunotherapy for Advanced Gastric Cancer	Recruiting		Sun Yat-sen University Cancer Center Guangzhou (Guangdong, China)	2019–2021 (e)
Study on the Adverse Drug Reactions (ADRs) of Apatinib and Their Biomarker Correlations	Recruiting	Drug: apatinib	Sun Yat-sen University Guangzhou Cancer Center (Guangdong, China)	2018–2019 (e)
Biomarker Study of PDR001 in Combination with MCS110 in Gastric Cancer	Recruiting	Drug: MCS110/PDR001 combination	Seoul National University Hospital (Seoul, Korea)	Jan 2019–Dec 2019 (e)
Identification of Biomarkers for Prediction of Response or Resistance Against Target Therapy in Gastric Cancer	Completed		University Cancer Center (Leipzig, Germany)	2014–2018
ctDNA for Prediction of Relapse in Gastric Cancer	Recruiting	ctDNA test	Medical Oncology, Sun Yat-sen University Guangzhou Cancer Center (Guangdong, China)	2016–2020 (e)

e: Estimated conclusion.

because many of the biomarkers used clinically nowadays for GC diagnosis and prognosis are also used for other malignancies, making the results less significant for GC patients.

The effect of GC biomarker expression on drug sensitivity is extremely promising because it would allow oncologists to mold the drug treatment of GC following the patient's molecular panel, making subsequent treatment more effective. A wider array of biomarkers needs to be tested against a wider array of drugs to determine sensitivity for different patients' unique molecular expression. Another promising field is one of developing drugs that specifically target common biomarkers expressed in GC tissue. Future drug studies should focus on these biomarkers for the development of more optimal GC diagnosis, prognosis and therapies.

Ten current studies investigating GC biomarkers are summarized in Table 2. These studies are present in the NIH's clinicaltrials.gov database as of January 2021 and investigate patients with GC only, and no other type of malignancies. Through this database, it is possible to observe the lack of research in regards to GC when compared with other malignancies. The term 'gastric cancer' produces a total of 1958 studies in the database, while the terms 'prostate cancer' and 'lung cancer' produce 4575 and 7345 results, respectively. When researching biomarkers specifically, only 267 studies are associated with the keywords 'gastric cancer biomarkers', while 847 results show up for 'prostate cancer biomarkers' and 1200 for 'lung cancer biomarkers'. Therefore the results for GC research are dismal and they translate into a reality where GC patients are faced with a less precise diagnosis, fewer therapy options and other clinical hurdles. More research in the area of this cancer with such high mortality and morbidity is crucial.

The authors speculate that GC research will increase in the next 5–10 years, due to the pressure of increasing risk factors for this malignancy: advancing age of the global population, increased Asian migration to Western countries and increased consumption of industrialized diets. We predict that research on diagnostic biomarkers of this malignancy will primarily focus on noncoding regions of the genome, because tumor-related genes are already more established in the literature. However, it is likely that a higher number of studies will show the impact of widely implementing clinical testing for the already established GC-related oncogenes and tumor suppressor genes. In terms of GC biomarkers for prediction of treatment outcomes, we speculate that this field will primarily focus

on how biomarkers influence immune-related therapies, given that immunotherapy is a pioneering area of research worldwide and is currently promising lower morbidity and higher efficacy in the fight against cancer.

Summary points

- Among biomarkers such as carcinoembryonic antigen, CA 19-9, AFP and CA-125, the one that showed most importance in relation to prognosis was carcinoembryonic antigen. When positive, overall survival was reduced by 25%.
- 70% of tumor suppressor genes were found to be hypermethylated in GC cases, associated with *H. pylori* and EBV infections.
- In cases when HER2 is found amplified, the median survival rate for gastric cancer is reduced.
- lncRNAs are important biomarkers. One example is *CASC15*, which is associated with tumorigenesis.
- Elevated levels of miRNAs such as miR-296-5p and miR-301 are associated with lower *CDX1* and *RNNX3* expression respectively, contributing to tumor proliferation and tissue invasion.

Author contributions

All authors contributed equally to manuscript preparation.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Data sharing statement

The authors state that their manuscript does not report the original results of a clinical trial or the secondary analysis of clinical trial data that has been shared with them.

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