Review

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Predictive biomarkers for targeted and cytotoxic agents in gastric cancer for personalized medicine

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Summary **Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer. The treatment of GC remains challenging as the outcomes achieved with surgery alone or adjuvant or neoadjuvant chemotherapy and radiotherapy are relatively poor. New treatment strategies are emerging and are being tested in solid tumors including GC. Over the past few years, the treatment of metastatic colorectal cancer (CRC) has made great advances, but strategies to manage GC have improved little. Multiple drug resistance is common in GC chemotherapy and targeted therapy; some patients appear to receive treatment that is suboptimal or even inefficacious. Unfortunately, there are few validated predictive biomarkers to guide the tailored treatment of GC. ToGA and AVAGAST are two phase III trials that tested the efficacy and safety of targeted agents in advanced gastric cancer (AGC), and results clearly indicated that patients need to be selected and that targeted agents are the best hope for better results. This review aims to provide an overview of potential predictive biomarkers for cytotoxic and targeted agents in GC.**

Keywords: Gastric cancer, biomarkers, chemotherapy, targeted therapy, personalized medicine, predictive marker

1. Introduction

Gastric cancer (GC) is the fourth most common cancer and the second leading cause of cancer related death worldwide, with an estimated 800,000 deaths caused by the disease (*1*). The incidence of gastric cancer varies widely by geographic region and is particularly common in East Asia (*2*). GC is primarily adenocarcinoma (approximately 95%), and GC can be further categorized into an intestinal form and a diffuse form based on its clinicopathologic features. The intestinal form develops amidst chronic atrophic gastritis, which is usually related to an *H. pylori* infection. In contrast, the diffuse form of gastric cancer is found in the proximal stomach and gastroesophageal junction (GEJ) and is common in populations suffering from chronic reflux disease (*3*).

Because of the early detection of GC and advances in chemotherapy and targeted therapy, the mortality rate of GC has decreased in most parts of the world. Surgical resection offers the best chance for curative therapy, but most newly diagnosed patients present with advanced and unresectable GC, and use of surgery alone or chemotherapy and radiotherapy to treat advanced gastric cancer (AGC) results in poor outcomes. The 5-year survival rate drops from 50-70% in early-stage GC to 4-10% in AGC. For these patients, chemotherapy is the primary treatment option (*4,5*). New treatment strategies for AGC, including targeted therapies, are emerging and being tested, but their efficacy is limited due to development of chemo-resistance.

Mounting evidence indicates that prognosis and treatment responses of a variety of cancers depend on the stage of the tumor as well as the phenotypic and genotypic characteristics of the tumor. Some patients appear to receive treatment that is suboptimal or even inefficacious. In personalized medicine, predictive biomarkers can be used to exclude therapies that the tumor will not respond to or to select therapies that the tumor is likely to respond to. Advances in the

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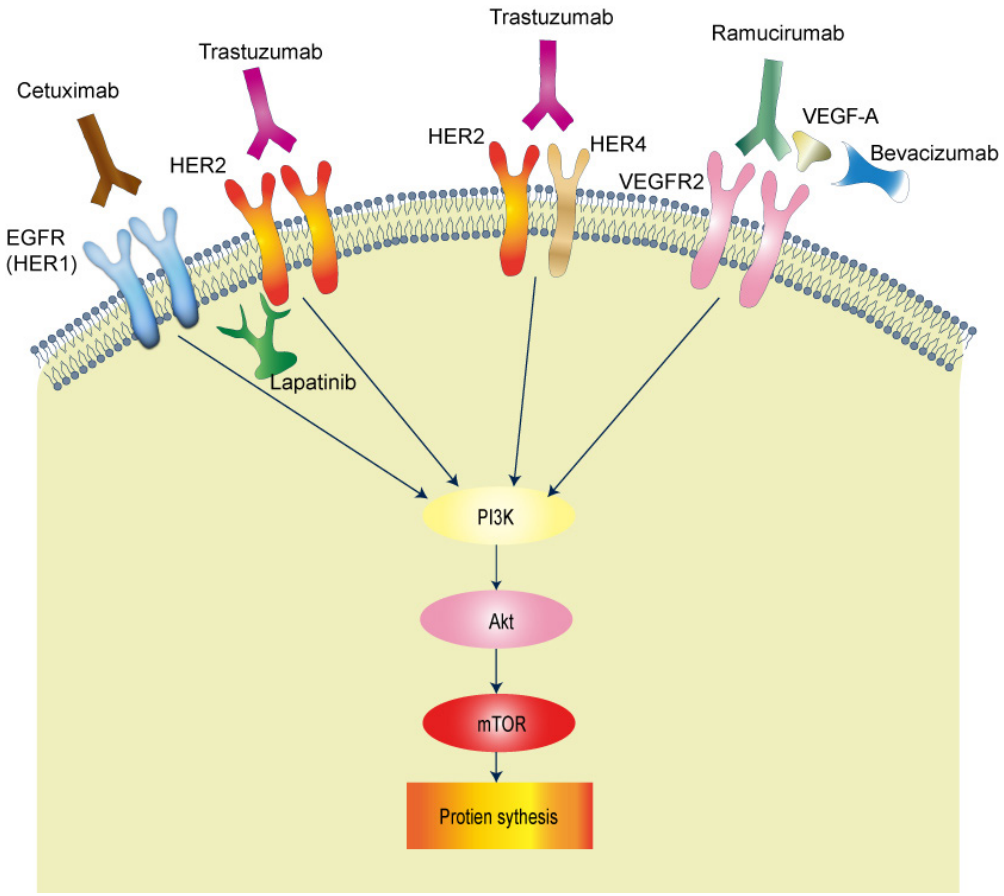

Figure 1. Molecular mechanisms of targeted therapy for GC. Targeted therapy with antibodies or specific small molecule inhibitors to treat GC involves overexpressed or amplified receptors, specific ligands, or receptor tyrosine kinases (RTKs). The targeted RTKs include EGFR (HER1), HER2, HER4, and VEGFR. VEGFR-A is the ligand targeted by bevacizumab. The activation of (RTK) signals via PI3K leads to the activation of mTOR.

identification and verification of prognostic biomarkers aids in early detection of GC and monitoring its recurrence, but the current understanding of predictive biomarkers is relatively limited. Personalized medicine based on predictive biomarkers is urgently needed to optimize patient selection and maximize treatment efficacy.

The aim of this review is to provide up-to-date information about predictive biomarkers for GC. Over the past few years, considerable effort has been devoted to identifying predictive biomarkers. Those biomarkers may include DNA repair enzymes, circulating tumor cells (CTCs), and microRNAs (miRNAs).

2. Predictive biomarkers for GC

2.1. *Human epidermal growth factor 2 (HER2)*

HER2 (encoded by *ERBB2*) is one of the four members of the human epidermal growth factor receptor family (EGFR or HER1, HER2, HER3 and HER4) in the receptor tyrosine kinase superfamily (Figure 1). *ERBB2* amplification and HER2 overexpression have been studied most often in breast cancer, and *ERBB2* amplification and HER2 overexpression are two of the most common biomarkers for GC. In breast cancer, amplification and overexpression of the *HER2* gene is associated with poor outcomes, higher mortality, and higher recurrence (*6*). *ERBB2* amplification or HER2 overexpression has been reported in 7-34% of patients with GC (*7-9*).

Results regarding the prognostic value of HER2 in GC are controversial. Some studies have reported that *ERBB2* amplification is associated with a poor prognosis and aggressive disease (*7,8,10*). However, other studies have reported finding no difference in prognosis between HER2-positive and HER2-negative tumors (*11,12*). Inhibition of HER2 has been induced as a potential targeted therapy for GC. Trastuzumab, a monoclonal antibody that targets HER2, inhibits HER2-mediated signaling and prevents cleavage of its extracellular domain (ECD).

The ToGA trial (NCT01041404) is a phase III international study that assessed the efficacy of a combination of trastuzumab with cisplatin plus 5-fluorouracil (5-FU) or capecitabine in patients with GC. The trial recruited 584 gastric/GEJ cancer patients with either HER2 overexpression (immunohistochemistry (IHC) 3+) or *ERBB2* gene amplification. The addition of trastuzumab to chemotherapy significantly increased the

response rate (47% *vs*. 35%), progression-free survival (PFS; 6.7 *vs*. 5.5 months), and overall survival (OS; 13.8 *vs*. 11.1 months). Moreover, the trial verified the predictive value of positivity for HER2. The median OS within the whole trastuzumab arm was 13.8 months, whereas it was 17.9 months in the "Very High HER2" group and 16 months in the "High HER2" group. Patients with FISH-positive and IHC 0/1+ GC did not benefit from trastuzumab treatment (*13*).

Results of several recent prospective studies also indicated that the level of *HER2* gene amplification significantly predicts sensitivity to therapy and OS in AGC treated with trastuzumab-based chemotherapy. CGOG1001 (NCT01364493) is a multicenter, prospective phase II study that evaluated the addition of trastuzumab to oxaliplatin/capecitabine in patients with chemotherapy-naive HER2-positive AGC. Patients with a HER2/CEP17 ratio of greater than five had an improved OS (20.9 *vs*. 19.5 months, $p = 0.001$) (*14*). Another prospective study in 90 patients with metastatic GC yielded similar results. In that study, a mean HER2/CEP17 ratio of 4.7 was identified as the optimal cutoff value distinguishing sensitive and refractory patients, and the optimal cutoff for predicting a survival longer than 12 months was 4.45 *(15*) . A cohort study of 126 patients with HER2-positive AGC treated with trastuzumab plus chemotherapy indicated that patients with HER2 IHC 3+ had a significantly longer OS than patients with IHC ≤ 2+. An HER2/CEP17 ratio of 4.48 was the optimal cutoff for predicting a longer OS (26.9 *vs*. 14.7 months; $p = 0.027$). In patients with IHC ≤ 2+, however, an HER2/CEP17 ratio of more than 3.69 and an *HER2* gene copy number (GCN) higher than 7.75 were positive predictive factors for better outcomes (*16*). Hence, HER2 and the HER2/CEP17 ratio can serve as predictive biomarkers for trastuzumab-targeted therapy in AGC.

Zhou *et al*. sought to identify blood-based predictive biomarkers for trastuzumab-treated AGC, and they found that the levels of HER2 ECD in serum were closely correlated with the HER2 status of tissue in AGC. There was a significantly better overall response rate and PFS for patients with abnormal baseline serum HER2 ECD than for patients with normal serum HER2 ECD. A change in serum HER2 ECD during chemotherapy was significantly correlated with a response to chemotherapy and PFS in patients with HER2-positive tumor tissue. These results substantiate the clinical utility of measuring serum HER2 ECD levels in patients with AGC. Baseline and early changes in serum HER2 ECD could be useful for monitoring clinical outcomes in patients with HER2-positive AGC receiving trastuzumab-combined chemotherapy (*17*).

A study investigated the role of the phosphoinositide 3-kinase (PI3K) pathway activation in HER2-targeted therapy in 48 patients receiving trastuzumab or lapatinib combination chemotherapy (*18*). Among the patients with responsive disease, the time to best response did not differ by phosphatase and tensin homolog (*PTEN*) status, but the duration of response was significantly shorter for patients with *PTEN* loss (median 4.2 *vs*. 6.1 months, $p = 0.04$). In addition, patients with *PTEN* loss had a significantly shorter PFS (median 4.9 *vs*. 7.3 months, $p = 0.047$). These findings suggest that a *PTEN* deficiency is an important predictive marker for early resistance to HER2 inhibitor treatment in patients with GC.

Lapatinib is a dual inhibitor of HER2 and EGFR tyrosine kinases (Figure 1). A Phase III trial (TyTAN) of lapatinib in combination with weekly paclitaxel versus weekly paclitaxel alone was conducted in patients with HER2-positive GC. The trial failed to find any improvement in OS. However, patients in the HER2 IHC 3+ subgroup who received lapatinib had a significantly prolonged survival (14 *vs*. 6.4 months; HR = 0.59; $p = 0.018$) and higher response rate (27% *vs*. 9%) (*19*).

2.2. *EGFR (HER1)*

EGFR is a member of the ERBB family of transmembrane RTKs. EGFR activation results in proliferation, angiogenesis, and migration *via* the MAP kinase and PI3K/AKT pathways (*20*). Cetuximab, a monoclonal antibody, attenuates the malignancy signal mediated by EGFR (Figure 1). A Phase III trial (EXPAND) involving 904 patients with GC indicated that addition of cetuximab to capecitabine/cisplatin as first-line treatment provided no additional benefit (*21*). A phase II trial (NCT00477711) indicated that EGFR overexpression predicted the efficacy of cetuximab combined with cisplatin and capecitabine in AGC or GEJ adenocarcinoma (*22*). Conversely, several phase II trials failed to verify the ability of EGFR IHC to predict the clinical response to cetuximab (*23,24*) or EGFR tyrosine kinase inhibitors (*25*) in GC.

Patients with metastatic colorectal cancer (mCRC) who responded to anti-EGFR treatment (cetuximab or panitumumab) had a significantly increased tumor *EGFR* GCN according to FISH (*26*). A study investigating the predictive role of *EGFR* gene amplification in patients with GC yielded similar results (*27*). The study used dual *in situ* hybridization to determine *EGFR* GCN gain (≥ 2.5 EGFR signals per cell), which it detected in 194 patients (22.7 %); *EGFR* GCN gain also predicted a poor prognosis. *EGFR* GCN gain is a more accurate prognostic biomarker than EGFR overexpression in patients with GC. Continued EGFR signaling might play a more important role in survival of *EGFR*-amplified GC cells than EGFR overexpression. Various oncogenic signals, such as *c-Jun* activation (*28*), may be involved in EGFR protein overexpression without gene amplification. A prospective study investigated EGFR expression and ligand levels in patients with GC and found that patients with EGFR expression and low ligand levels may have better outcomes with cetuximab/mFOLFOX6

treatment (*29*). Moreover, the study found that ligand levels increased when disease progressed in 7 of 8 patients with EGFR expression and low baseline ligand levels. Drawing any conclusions from that study is difficult due to the small number of evaluable patients (*n* = 38), but evaluation of *EGFR* amplification in large-scale trials might yield promising results.

Kirsten rat sarcoma viral oncogene homolog (*KRAS*) mediates the transduction of signals between *EGFR* and the nucleus, and *KRAS* mutations may serve as a negative predictor of the response of CRC to cetuximab *(30*). However, *KRAS* mutations have not been fully evaluated as predictive markers of EGFR-targeted therapy in patients with GC due to their low frequencies in patients with GC. Results of *in vitro* experiments indicated that *KRAS* mutations were associated with cetuximab resistance in 5 GC cell lines (*31*). The growth of GC cells with wild-type *KRAS* and xenografted GC cells with a *KRAS* (G→A) mutation was significantly inhibited by cetuximab. However, apoptosis was induced in GC cells with wild-type *KRAS* but not in xenografted GC cells with a *KRAS* (G→A) mutation after cetuximab treatment. A *KRAS* (G→A) mutation does not affect the anti-cancer efficacy of cetuximab in GC cell lines (*32*). These findings imply that *KRAS* point mutations might predict the response of GC to cetuximab.

2.3. *Vascular endothelial growth factor (VEGF)*

The role of VEGF has been extensively studied in several cancers, including GC. Vascular endothelial growth factor A (VEGF-A) and its receptors (VEGFRs) play an important role in angiogenesis leading to tumourigenesis and metastasis. Expression of VEGF-A was reported in 40% of patients with GC and expression of VEGFR was reported in 36% (*33*). VEGF-A expression in a tumor and serum correlates with a lack of a response to chemotherapy, as well as with more aggressive behavior of the tumor, both in resectable GC and AGC.

Bevacizumab, a monoclonal antibody against VEGF-A, has been the anti-angiogenesis agent most often used to decrease the vascular supply to a tumor and slow metastasis (Figure 1). Bevacizumab has successfully improved OS in advanced CRC (*34*). A phase III randomized, double-blind, contrast study (AVAGAST, NCT00548548) tested the efficacy of first-line bevacizumab in 774 unselected patients with GC (*35*). Patients were treated with capecitabine and cisplatin in combination with either bevacizumab or a placebo. The median rate of OS was 10.1 months for the placebo group and 12.1 months for the bevacizumab group (HR = 0.87; $p = 0.1002$), failing to meet the primary endpoint. Nevertheless, the addition of bevacizumab to chemotherapy (capecitabine/cisplatin) improved PFS and the response rate compared to chemotherapy plus a placebo. Moreover, high levels of plasma VEGF-A predicted an improvement in OS (HR = 0.72; 95% CI, 0.57 to 0.93), and low expression of tumor neuropilin-1 also predicted an improvement in OS (HR = 0.75; 95% CI, 0.59 to 0.97) in the bevacizumab group. In contrast, the predictive value of neuropilin-1 was not noted in another phase II trial (*36*). According to an analysis of AVAGAST subgroups, VEGF-A and neuropilin-1 had the ability to predict OS only in non-Asian patients (*37*). Another trial found a similar ethnic difference since VEGR-A levels were independent predictors of OS in Caucasians with GC but not in Asians with GC (*38*). Therefore, the predictive role of VEGF-A might depend on ethnicity and the type of tumor.

2.4. *Thymidylate synthase (TS)*

TS provides an effective predictor of the response to chemotherapy with 5-FU. One mechanism by which 5-FU displays anticancer action is through the inhibition of TS, which is a key enzyme in the process of DNA replication and repair. Most GC chemotherapy regimens include 5-FU, so responsiveness to chemotherapy may, in theory, be affected by TS status.

Patients with GC have significantly higher levels of TS mRNA in plasma than do healthy controls, and levels of TS mRNA in plasma were significantly correlated with levels of TS mRNA in tumor tissues *(39*). Low levels of expression of TS mRNA in a tumor and plasma were significantly correlated with raltitrexed sensitivity. When expression of TS mRNA in a tumor and plasma was used to predict the response to chemotherapy, raltitrexed sensitivity predicted on the basis of levels of TS mRNA in plasma had a sensitivity of 82% and an accuracy of 60% while sensitivity predicted on the basis of levels of TS mRNA in a tumor had a sensitivity of 70% and an accuracy of 68% (*39*). Endoscopic biopsies in patients with AGC have also indicated that levels of expression of TS were significantly higher in a tumor than in normal tissue and significantly lower in S-1/cisplatin responders than in S-1/cisplatin non-responders. Interestingly, a significant increase in TS expression was detected in several patients, who changed from "responders" to "non-responders" after chemotherapy (*40*). An *in vitro* study has found that levels of TS in plasma and tissue are negatively associated with pemetrexed sensitivity (*41*).

2.5. *DNA repair enzymes*

X-ray repair cross complement group 1 (XRCC1), excision repair cross-complementing 1 (ERCC1), and BRCA1, known as DNA repair enzymes, have recently garnered attention because of their role in predicting the response to chemotherapy in patients with GC. XRCC1 and ERCC1 expression was significantly

Table 1. MiRNAs as predictive biomarkers in GC*

MiRNA	Year	Specimens (up or down-regulated in chemo-resistant specimens)	Target	Function	*Ref.*
MiR-125a-5p	2011	Tissues and cell lines	*ERBB2*	Enhances antitumor efficacy in combination with trastuzumab	(*60*)
MiR-27a	2011;2014	Cell lines and mouse model; Plasma	-	Modulates MDR; Predicts resistance to fluoropyrimidine-based chemotherapy	(*61,62*)
MiR-497	2012	Cell lines (down-regulated)	*Bcl-2*	Modulates MDR	(*63*)
MiR-200bc/429 cluster	2012	Cell lines (down-regulated)	Bcl-2,XIAP	Modulates MDR	(*64*)
MiR-17-5p,MiR-20a	2012	Plasma and mouse model	-	Modulates chemotherapeutic effects	(*65*)
miR-21	2013	Cell lines (up-regulated)	*PTEN*	Modulates MDR	(*66*)
MiR-106a	2013	Cell lines (up-regulated)	*PTEN*	Modulates MDR	(*67*)
MiR-1271	2014	Cell lines (down-regulated)	IGF1R, IRS1, mTOR, and BCL-2	Modulates MDR	(*68*)
MiR-429	2015	Tissues and cell lines	Bcl-2	Modulates chemotherapeutic effects	(*69*)
MiR-218	2015	Cell lines (down-regulated)	SMO	Inhibits MDR	(*70*)
MiR-143	2015	Cell lines (down-regulated)	IGF1R and BCL-2	Modulates MDR	(*71*)
MiR-103/107	2015	Cell lines (down-regulated)	caveolin-1	Modulates MDR	(*72*)
MiR-223	2015	Cell lines	FBXW7	Modulates trastuzumab-induced apoptosis	(*73*)
MiR-26a	2015	Cell lines (down-regulated)	NRAS and E2F2	Modulates MDR	(*74*)
MiR-23b-3p	2015	Cell lines and mouse model (down-regulated)	ATG12 and HMGB2	Modulates MDR	(*75*)
MiR-1284	2016	Tissues and cell lines (down-regulated)	EIF4A1	Modulates MDR	(*76*)
MiR-375	2016	Cell lines (down-regulated)	ERBB2	Modulates MDR	(*77*)
MiR-181	2016	Cell lines and mouse model (down-regulated)	ATG5	Modulates autophagy and chemo-resistance	(*78*)
MiR-27b	2016	Cell lines and mouse model	CCNG1	Modulates MDR	(*79*)

*XIAP: X-linked inhibitor of apoptosis protein; IGF1R, type 1 insulin-like growth factor receptor; IRS1, insulin receptor substrate 1; BCL-2, B cell leukemia/lymphoma 2; SMO, smoothened, frizzled class receptor; FBXW7, F-box/WD repeat-containing protein 7; NRAS, neuroblastoma RAS viral (v-ras) oncogene homolog; E2F2, E2F transcription factor 2; ATG12, autophagy related 12; HMGB2, high mobility group box 2; EIF4A1, eukaryotic translation initiation factor 4A1; ATG5, autophagy related 5; CCNG1, cyclin G1.

downregulated in GC tissue and XRCC1 and ERCC1 have been identified as negative markers of OS in most studies (*42-45*). ERCC1 mRNA overexpression is associated with an unfavorable response to regimens with platinum agents (*46-48*). In contrast, the *XRCC1* and *ERCC1* genes are not able to predict the disease control rate (*49,50*). Two studies failed to verify the ability of tumor ERCC1 expression to predict the clinical response or survival of patients with AGC (*51,52*). Due to these conflicting results, the predictive role of XRCC1 and ERCC1 needs to be verified in large-scale prospective clinical trials.

Studies of *XRCC1* and *ERCC1* gene polymorphisms as a predictor of the response to chemotherapy have yielded encouraging results. In GC, the specific presence of an A/G polymorphism in *XRCC1* at codon 399 and the combination of the A/G polymorphism in *XRCC1* at codon 399 and a C/T polymorphism in ERCC1 at codon 118 is a predictor of median OS for patients receiving oxaliplatin/5-FU-based chemotherapy (*49,50*). A polymorphism in *XRCC1* at codon 194 (Arg>Trp) was correlated with a better response to chemotherapy (*53*). The *XRCC1* 194 C/T genotype could be a modest predictor of AGC response in patients treated with taxane and cisplatin (*54*). Retrospective studies found that the *ERCC1* rs3212986 and rs11615 polymorphisms influenced the response to chemotherapy and the OS of patients with GC (*55-58*).

Germline mutations in *BRCA1* are associated with an increased risk of developing breast cancer, ovarian cancer, gastric cancer, and other types of cancers. *BRCA1* heterozygosity has been found to cause a predisposition to GC (*59*). A low level of *BRCA1* mRNA in a tumor was associated with an increased response rate (*59*). However, conflicting results have been reported since BRCA1 levels in plasma and a tumor were positively associated with docetaxel sensitivity. The *BRCA1* TT genotype could be a modest predictor of AGC response in patients treated with taxane and cisplatin (*54*). *BRCA1* mRNA and *BRCA1* polymorphisms may be potential predictive biomarkers for chemotherapy.

2.6. *miRNAs*

miRNAs are a relatively novel class of regulatory molecules that control the translation and stability of mRNAs on a post-transcriptional level *via* interaction with the 3'-untranslated region (UTRs) of target mRNAs, eventually leading to destabilization and/or inhibition of their translation. Multidrug resistance (MDR) correlates with treatment failure and a poor

prognosis among patients with GC. Aberrant patterns of miRNA expression have been implicated in MDR in GC cells. miRNAs could potentially be used to predict the response to chemotherapy. The current literature describing the impact of miRNAs on the prediction of and changes in sensitivity to anticancer treatment is summarized in Table 1.

An association between aberrant patterns of miRNA expression in GC and MDR has been noted *in vivo* and *in vitro*. Expression of 12 miRNAs (miR-497 (*63*), miR-200bc/429 cluster (*64*), miR-1271 (*68*), miR-218 (*70*), miR-143 (*71*), miR-103 (*72*), miR-107 (*72*), miR-26a (*74*), miR-23b-3p (*75*), miR-1284 (*76*), miR-375 (*77*), and mi-181 (*78*)) was downregulated in GC cells. Overexpression of these miRNAs sensitized tumors to anticancer drugs. miR-21 (*66*) and miR-106a (*67*) were found to be up-regulated in chemo-resistant GC cell lines. Overexpression of miR-21 and miR-106a significantly decreased the antiproliferative effects of anti-cancer drugs and the apoptosis they induced, while knockdown or suppression of miR-21 and miR-106a dramatically increased the cytotoxicity of anti-cancer drugs. Down-regulation of miR-27a conferred sensitivity to chemotherapy in GC cells (*61*). Moreover, patients with up-regulated levels of miR-27a expression had a significantly worse OS than patients with lower levels of miR-27a expression ($p = 0.024$). miR-27a is a potential biomarker to predict resistance to fluoropyrimidine-based chemotherapy in patients with metastatic or recurrent GC (*62*). Ectopic miR-27b in GC tumors led to increased sensitivity to chemotherapy *in vitro* and *in vivo* (*79*). The levels of miR-17-5p/20a in serum decreased markedly in treated mice with a decreased tumor volume (*65*). Suppression of miR-429 in GC cells promotes Bcl-2-mediated cancer cell survival in response to chemotherapy-induced cell death. Restored levels of miR-429 expression may enhance cancer apoptosis in GC cells during chemotherapy (*69*). These findings suggest that these miRNAs have the potential to be molecular markers of pathological progression, to predict prognosis, and to monitor the response of GC to chemotherapy.

miRNAs have also been found to be potential biomarkers for targeted therapy. *In vitro* assays indicated that *ERBB2* is a direct target of miR-125a-5p; miR-125a-5p potently suppresses the proliferation of GC cells and it suppresses that proliferation even more so in combination with trastuzumab (*60*). Overexpression of miR-223 decreased the sensitivity of GC cells to trastuzumab while suppression of miR-223 restored the sensitivity of GC cells to trastuzumab. Moreover, overexpression of miR-223 significantly suppressed trastuzumab-induced apoptosis (*73*).

Despite the promising results described here, research on miRNAs is still in its infancy. Studies of miRNAs in GC are limited and thus far only describe experiments and clinical observations. Unfortunately, few clinical trials have involved patients with GC. From a clinical point of view, there are no reliable biomarkers available that allow the prediction of the response to chemotherapy (*80*).

2.7. *CTCs*

Advances in techniques have allowed the detection and characterization of CTCs (and even rare types of those cells) in peripheral blood. Metastasis of a solid tumor requires tumor cells to enter the circulation and travel to distant sites to establish a metastatic focus. Studies have focused on the potential role of CTCs in metastasis. Different methods have been used to detect and isolate CTCs. These include RT-PCR of whole blood, plasma, and sera, flow cytometry, and the related technique of immunomagnetic separation.

Analysis of CTCs has been used to predict prognosis, monitor the response to treatment, and monitor a relapse in breast cancer, mCRC, and melanoma (*81-83*). The role of CTCs in GC was evaluated in a phase II study involving patients with advanced HER2-negative GC or GEJ adenocarcinoma. The presence of CTCs at the baseline was found to be strongly predictive of PFS (HR = 3.8; $p = 0.007$) and OS (HR = 3.4; $p = 0.014$) (*84*). Patients who were CTC-positive at the baseline had a significantly shorter median PFS and OS (*85*). These findings suggest that a favorable clinical response depends significantly on negativity for CTCs.

Measuring the CTC count to monitor the response to treatment is an attractive area of research. The CTC count was found to decrease on day 16 following chemotherapy and then increase again during the chemo-resistant phase in AGC (*86*). Matsusaka *et al.* used immunomagnetic separation to isolate CTCs and they measured the CTC count in whole blood at the baseline and 2 and 4 weeks after initiation of chemotherapy (*87*). Patients with AGC receiving S-1-based or paclitaxel chemotherapy with ≥ 4 CTCs at 2 weeks and 4 weeks had a shorter median PFS and OS. An epithelial-to-mesenchymal transition (EMT) was also evident in a few cells of primary tumors and more so in CTCs from the blood of patients with GC, so this phenomenon might be used to monitor the response to treatment (*88*). HER2-positive CTCs were effectively eliminated by HER2-targeted therapy in patients with HER2-positive AGC. Determining the number of copies of chromosome 8 in CTCs provides a potential approach to predicting chemotherapeutic efficacy and monitoring chemo-resistance (*89*). Thus, the CTC count may serve as an early biomarker that allows the evaluation of therapeutic efficacy.

3. Conclusion and prospects for the future

This review has described most of the predictive markers that guide the choice of the most suitable

therapy for individual patients with GC. Compared to major developments in targeted treatment of mCRC in recent years, strategies to manage GC have improved little. Anti-HER2 therapy is the only targeted therapy that is currently accepted as standard treatment for GC, and very high levels of HER2 expression predict which patients will benefit from this therapy.

TNM staging has been a vital tool to assess prognosis and predict the need for systemic treatment of resectable GC. However, several studies have highlighted the importance and necessity of genotypic and phenotypic classification of GC to facilitate patient treatment. The success of the ToGA trial and, more recently, the failure of bevacizumab in a large phase III study (*37*) in unselected patients with GC clearly show that patients need to be selected and that patients selection is the best hope for better results of targeted agents. Potential tumor and blood-based predictive biomarkers need to be further investigated for appropriate patient stratification and personalized oncologic treatment.

Since MDR is a common and complex phenomenon attributed to crosstalk and feedback between multiple signaling pathways, a single biomarker may have limited power to predict a clinical response. Rapid advancements in sequencing and mass spectrometry techniques have allowed simultaneous evaluation of multiple signaling pathways in specimens. An evaluation of multiple signaling pathways may help with efforts to improve personalized treatment with targeted agents and, possibly, cytotoxic agents (*90,91*).

Most clinically actionable targets are relatively infrequent in GC. In order to evaluate the predictive value of potential markers, a combined effort is needed to procure an adequate number of pretreatment tumor specimens to ensure that projects to identify biomarkers have robust statistical power. Novel techniques may help in the early evaluation of tumor response after anti-cancer treatment. *In vivo* apoptosis imaging using Apopep-1 (*92*) has been found to be a sensitive and predictive tool for early determining of the response of GC after anti-cancer treatment.

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