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Gastrointestinal Cancer

How Prognostic and Predictive Biomarkers Are Transforming Our Understanding and Management of Advanced Gastric Cancer

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. Gastric cancer • Biomarkers • Prognostic • Predictive • HER2

ABSTRACT

Background. Gastric cancer (GC) is the second leading cause of cancer death worldwide. GC is a heterogeneous disease in terms of histology, anatomy, and epidemiology. There is also wide variability in how GC is treated in both the resectable and unresectable settings. Identification of prognostic and predictive biomarkers is critical to help direct and tailor therapy for this deadly disease.

Methods. A literature search was done using Medline and MeSH terms for GC and predictive biomarkers and prognostic biomarkers. The search was limited to human subjects and the English language. There was no limit on dates. Published data and unpublished abstracts with clinical relevance were included.

Results. Many potential prognostic and predictive biomarkers have been assessed for GC, some of which are becoming practice changing. This review is focused on clinically relevant biomarkers, including EGFR, HER2, various markers of angiogenesis, proto-oncogene MET, and the mammalian target of rapamycin.

Conclusion. GC is a deadly and heterogeneous disease for which biomarkers are beginning to change our understanding of prognosis and management. The recognition of predictive biomarkers, such as HER2 and vascular endothelial growth factor, has been an exciting development in the management of GC, validating the use of targeted drugs trastuzumab and ramucirumab. MET is another potential predictive marker that may be targeted in GC with drugs such as rilotumumab, foretinib, and crizotinib. Further identification and validation of prognostic and predictive biomarkers has the potential transform how this deadly disease is managed. ***The Oncologist*** 2014;19:1046–1055

Implications for Practice: The identification of prognostic and predictive biomarkers has the potential to change how gastric cancer is understood and managed. Although many biomarkers have been investigated in gastric cancer, few have proven to have practice-changing implications. This article reviews the data behind clinically relevant biomarkers for advanced gastric cancer. Of particular interest is the recognition of HER2 and vascular endothelial growth factor as predictive markers, which has validated the use of trastuzumab and ramucirumab in clinical practice. MET is another up and coming biomarker, with multiple potential targeted therapies. The discovery and validation of biomarkers in gastric cancer is essential to aid our understanding of the pathogenesis and management of this deadly disease.

INTRODUCTION

Gastric cancer (GC) incidence has been decreasing over the past few decades; however, it remains the second leading cause of cancer death worldwide [1, 2]. In 2014, it is estimated that there will be 22,200 new cases in the U.S., with 10,990 deaths due to GC that same year [3]. GC is now relatively rare in Western countries, but rates remain high in areas of Eastern Europe, Asia, and South and Central America [2]. The majority of GCs are adenocarcinomas, which are divided histologically into an intestinal or diffuse type and anatomically into cardia or noncardia cancers. Pathogenesis and prognosis differ based on GC subtype. Intestinal-type GC, for example, is more common in Asia and tends to be associated with *Helicobacter pylori* infection [4]. Diffuse-type GC seems to have a worse prognosis [5, 6]. Rates of noncardia GC are decreasing worldwide; however, in countries where GC remains common, noncardia GC persists, whereas proximal cancers are more common in North America and Europe [2]. Proximal GC is associated with gastroesophageal reflux disease and shares similarities with malignancies of the esophagus or gastroesophageal junction (GEJ) [4]. Despite the geographical, histological, and anatomical heterogeneity of GC, it is treated as one disease entity and, unfortunately, the outcomes are poor.

Further evidence of the heterogeneity of GC is demonstrated by variation in survival by geographical location. The 5-year survival rate for GC in the U.S. is only 26.9% [7], and survival rates are significantly higher in Asian populations [8–10]. Although there have been advances in the management of GC, surgical resection remains the only chance of cure.

CME

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It is unclear if the difference in survival by geographic location is due to a difference in biology or a difference in management, including surgical technique. Historically, in North America and Europe, adequate surgical resection consisted of a standardized limited (D1) lymphadenectomy, after the Dutch Gastric Cancer Group trial [11] and the UK Medical Research Council trial [12] showed no improvement in survival with standardized extended (D2) lymphadenectomy over D1 lymphadenectomy. In fact, these two studies showed increased morbidity and mortality with D2 lymphadenectomy. However, based on retrospective data [13, 14] suggesting improved survival with no increased mortality, D2 lymphadenectomy has long been the standard in Japan. Long-term follow-up from the Dutch Gastric Cancer Group trial suggests that D2 lymphadenectomy does indeed decrease locoregional recurrences and GC-related deaths and that surgical morbidity and mortality can be decreased by using a spleen-preserving D2 procedure [15].

Despite the bleak outcomes in GC, the past two decades have seen improvements in the systemic management of GC, including the adoption of adjuvant therapy. The Intergroup 0116 trial, conducted in a North American population, showed a decrease in locoregional and distant relapses with adjuvant chemoradiotherapy for patients with resectable adenocarcinoma of the stomach or GEJ [16]. An updated analysis reported 10-year median follow-up with median overall survival (OS) of 35 months in the adjuvant chemoradiotherapy group compared with 27 months in the surgery-alone group [17]. Even greater benefits with adjuvant chemotherapy have been demonstrated in Asian populations. S-1, an oral fluoropyrimidine, was shown to improve relapse-free survival and OS in Japanese patients after D2 lymphadenectomy [18]. Adjuvant capecitabine and oxaliplatin (the CAPOX regimen) were also shown to improve disease-free survival in South Korean, Chinese, and Taiwanese patients with stage II and III GC who underwent D2 resection [10].

Another option shown to improve survival of patients with GC is the administration of perioperative chemotherapy. This was demonstrated in the Medical Research Council Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial, which showed that the addition of perioperative epirubicin, cisplatin, and infusional fluorouracil (ECF) in Western patients with resectable adenocarcinoma of the stomach, GEJ, or lower esophagus resulted in 5-year OS of 36% compared with 23% in the control arm [19]. Similarly, a phase III trial of perioperative cisplatin and infusional fluorouracil (CF) compared with surgery alone showed similar 5-year OS and an increased R0 resection rate in an Asian population [20]. None of these treatment options has been proven to be superior; therefore, these options are all viable for increasing the chance of cure for patients with resectable gastric cancer.

There is no internationally recognized standard or superior regimen for the management of advanced GC. In order to improve outcomes, it is critical to understand the molecular pathogenesis of GC and to identify biomarkers of prognostic or predictive significance.

Unfortunately, most patients with GC present with advanced and unresectable disease [7], and of those who present at an earlier stage, many experience relapsed or metastatic disease. The primary treatment options for advanced or metastatic GC include best supportive care (BSC) and chemotherapy. A Cochrane meta-analysis showed a significant survival benefit when patients with advanced GC were treated with chemotherapy compared with BSC [21]. Combination chemotherapy provides a survival benefit over single-agent chemotherapy, with a hazard ratio (HR) of 0.82 (95% confidence interval [CI]: 0.74–0.90), but at the cost of increased side effects [21]. This meta-analysis also examined three-drug chemotherapy regimens and showed a benefit to both adding an anthracycline to CF-containing regimens (HR: 0.77; 95% CI: 0.62–0.95) and adding cisplatin to fluorouracil/anthracycline-containing regimens (HR: 0.83; 95% CI: 0.76–0.91). Individually, the three studies looking at adding an anthracycline to CF-containing regimens were negative for a survival benefit [22–24]; however, the numbers of patients enrolled in two of these studies were very small (47 and 120, respectively). When the data from all three studies were pooled in a meta-analysis, the HR for death when an anthracycline was added to CF-containing regimens was 0.77, with an average of a 2-month survival benefit. Four anthracycline-containing triplet regimens (ECF; epirubicin, cisplatin, and capecitabine [ECX]; epirubicin, oxaliplatin, and fluorouracil [EOF]; and epirubicin, oxaliplatin, and capecitabine [EOX]) were compared in the Randomized ECF for Advanced and Locally Advanced Esophageal Cancer-2 (REAL-2) study and were found to have similar OS [25], suggesting that capecitabine can be substituted for fluorouracil and oxaliplatin substituted for cisplatin. However, a meta-analysis of the REAL-2 and ML17032 trials suggested that capecitabine combination therapy is superior to fluorouracil combinations [26]. The V325 study also confirmed the benefit of triplet chemotherapy for advanced GC and showed an additional survival benefit when a three-drug regimen of docetaxel, cisplatin, and fluorouracil (DCF) was administered, compared with CF; however, again, that benefit was at the expense of increased toxicity, particularly neutropenia [27]. The Cochrane meta-analysis and the V325 study clearly show a small but significant benefit to the use of a three-drug chemotherapy regimen over a two-drug regimen, albeit at the cost of increased toxicity. Which drugs should be used (DCF versus an anthracycline-containing regimen) is less clear.

There is no internationally recognized standard or superior regimen for the management of advanced GC. In order to improve outcomes, it is critical to understand the molecular pathogenesis of GC [28] and to identify biomarkers of prognostic or predictive significance. With the cost of new targeted therapies, it is important to identify patients with biomarkers that predict response to these agents. Ideally, physicians will be able to tailor therapy toward individual patients according to biomarkers suggesting increased likelihood of response, thus improving outcomes for those with GC and sparing patients without these predictive biomarkers from the toxicities of ineffective therapies. In contrast, prognostic markers are used to identify patients with poor outcomes or a more aggressive disease course [29]. This review will focus on therapies directed at clinically relevant prognostic and predictive biomarkers in GC.

Vascular Endothelial Growth Factor

Angiogenesis is the process by which blood vessels sprout in an uncontrolled manner from pre-existing vasculature [30], promoting tumor growth, development, and metastasis [30–35]. The members of the vascular endothelial growth factor (VEGF) family—VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PGF)—are involved in continually encouraging angiogenesis in neoplasms [30, 35]. Binding of VEGF to one of three VEGF receptors results in receptor dimerization, phosphorylation, and downstream stimulation of cell growth and angiogenesis [36–38].

VEGF is expressed in 42%–49% of GCs [39, 40]. Higher serum VEGF levels have been associated with increased disease burden [41] and worse clinical outcomes [39, 40, 42–44] in GC. A prospective biomarker analysis [45] showed that higher levels of VEGF-A are associated with worse survival [46]. Low levels of neuropilin-1, a transmembrane glycoprotein involved in angiogenesis as a coreceptor for VEGF ligands [47], is associated with poor prognosis [46].

VEGF and the VEGF receptors (VEGFRs) have also been investigated as predictive biomarkers in GC. Multiple phase II studies have shown promising results when bevacizumab, a humanized monoclonal antibody against VEGF-A, was added to chemotherapy backbones including irinotecan and cisplatin [48] and docetaxel and oxaliplatin [49].

The phase III randomized controlled Avastin in Gastric Cancer (AVAGAST) trial studied the effect on OS when bevacizumab was added to capecitabine and cisplatin in the first-line setting for patients with advanced GC [45]. Although there was an increased response rate (RR) and an increase in median progression-free survival (PFS) in the bevacizumab-plus-chemotherapy group compared with the chemotherapy-alone group (RR: 46% versus 37%; PFS: 6.7 months versus 5.3 months), there was no statistically significant difference in the median OS (12 months versus 10 months). A preplanned subgroup analysis investigated biomarkers, including VEGF, VEGFR-1, VEGFR-2, and neuropilin-1 [46], to see if it was possible to identify which tumors were more responsive to bevacizumab. Interestingly, at baseline, biomarker levels were different based on geographic location. Higher serum VEGF-A, a poor prognostic marker, was seen in patients from non-Asian countries, whereas higher neuropilin-1 levels, a good prognostic marker, were seen in patients from Asia. Patients with high baseline plasma levels of VEGF-A in the bevacizumab-plus-chemotherapy group had improved OS compared with those with low VEGF-A levels, although the difference did not reach statistical significance. Non-Asian patients and patients with the highest plasma VEGF-A levels appeared to receive the greatest benefit from bevacizumab plus chemotherapy. Low neuropilin-1 levels were also associated with non-statistically significant prolonged survival with bevacizumab plus chemotherapy. These results are promising, but further studies are needed to identify and confirm which patient populations with GC benefit from the addition of bevacizumab to chemotherapy.

Ramucirumab is a fully human monoclonal antibody against VEGFR-2. It has been shown to have a role as both a single agent and in combination with chemotherapy in the second-line treatment of patients with metastatic GC. As a single agent, ramucirumab increased median OS to 5.2 months compared with 3.8 months in patients who received placebo [50]. The results of the phase III study of Paclitaxel With or Without Ramucirumab in Metastatic Gastric Adenocarcinoma (RAINBOW) trial [51] comparing second-line ramucirumab plus paclitaxel and placebo plus paclitaxel were recently presented. Patients who received ramucirumab plus paclitaxel had a median OS of 9.63 months compared with 7.36 months for those in the control arm. There was more neutropenia, leucopenia, and hypertension among patients who received ramucirumab. These results are a promising development for patients with advanced GC; however, the patients in the RAINBOW trial were required to have excellent Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤ 1, which is rare in patients who progress after first-line chemotherapy for metastatic GC. Regulatory approval of ramucirumab is currently being sought in many countries around the world.

Small molecule tyrosine kinase inhibitors (TKIs) have also been investigated in phase II studies. Sorafenib is an oral TKI that inhibits multiple targets, including VEGFR-2 and VEGFR-3. Used in combination with docetaxel and cisplatin in the first-line metastatic setting, the RR was 41% [52]. Another phase II study investigating the role of sorafenib in addition to capecitabine and cisplatin in the first-line setting (ClinicalTrials.gov identifier NCT01187212) is currently under way. Sorafenib does not appear to have a role either as a single agent [53] or in combination with chemotherapy [54] in the second-line setting. Sunitinib is an oral multitargeted TKI with targets that include VEGFR-1, VEGFR-2, and VEGFR-3. A phase II study showed that use of single-agent sunitinib in patients with advanced GC who had failed first-line chemotherapy resulted in an RR of only 3% [55]. Although small molecule TKIs are promising for the treatment of many other malignancies, they have not yet proven successful for the management of advanced GC.

Human Epidermal Growth Factor

The human epidermal growth factor receptor (HER) family involves four transmembrane tyrosine kinase receptors: HER1 (ErbB1, epidermal growth factor receptor [EGFR]), HER2 (ErbB2), HER3 (ErbB3), and HER4 (ErbB4) [56–59]. When a ligand binds to and activates the extracellular domain of the HER1, HER3, or HER4 receptors, they undergo dimerization, resulting in phosphorylation of intracellular tyrosine kinases, leading to downstream activation of pathways involved in cell growth, proliferation, and survival [56, 60, 61]. HER2 is activated through heterodimerization with other members of the HER family [56].

Epidermal Growth Factor

EGFR is expressed in approximately one-third of gastric carcinomas [40, 62], and there is evidence that high copy number is associated with worse outcomes in GC [40, 63, 64]. Other studies suggest that EGFR expression may be either a good prognostic factor [65] or of no prognostic significance at all [66].

EGFR has also been explored as a predictive biomarker in GC. Cetuximab, a chimeric monoclonal antibody targeting the extracellular domain of EGFR, binds to EGFR, blocking binding

of EGF and transforming growth factor-α (TGF-α) and preventing receptor dimerization and downstream activation [67]. A phase II study of single-agent cetuximab in patients previously treated for GC showed no benefit, with an RR of 3% [68]. The phase III Erbitux (cetuximab) in Combination With Xeloda (capecitabine) and Cisplatin in Advanced Esophagogastric Cancer (EXPAND) trial randomized patients with unresectable or metastatic GC to capecitabine and cisplatin with or without cetuximab [69]. The outcome was disappointing because the addition of cetuximab to chemotherapy did not significantly improve the primary endpoint of PFS. This trial was not limited to patients with tumors expressing EGFR. When EGFR immunohistochemistry (IHC) score was assessed retrospectively, the exploratory analysis suggested no relationship between EGFR IHC score and PFS or OS in either treatment group.

Other monoclonal antibodies targeting EGFR include the humanized antibody, matuzumab, and the fully human antibody, panitumumab. Matuzumab plus chemotherapy did not improve the overall RR in a phase II trial for patients with metastatic GC compared with chemotherapy alone [70]. A study of panitumumab plus EOX versus EOX alone showed worse OS in the panitumumab group [71].

EGFR TKIs have also shown minimal efficacy in GC. In a phase II study of single-agent gefitinib, no patients with GC had a radiological response to gefitinib, and tumor staining for EGFR and TGF-α had no predictive value [72]. A study of erlotinib also showed a small RR in patients with GEJ tumors, and no response in patients with gastric tumors [73].

These results highlight that the prognostic role of EGFR is unclear and that EGFR does not appear to be a predictive biomarker for GC.

HER2

HER2 overexpression or gene amplification is variable in GC and ranges from ~10% to 30% [62, 74–77], depending on tumor location and subtype. *HER2* amplification is more common in intestinal-type tumors and in tumors located in GEJ [75, 76, 78]. Of the patients enrolled in the Trastuzumab for Gastric Cancer (ToGA) trial, which assessed trastuzumab with cisplatin and a fluoropyrimidine (5-fluorouracil [5-FU] or capecitabine) in comparison to cisplatin and a fluoropyrimidine alone in patients with advanced GC, 22.1% of patients were HER2 positive, with higher rates of HER2 positivity in GEJ tumors (33.2%) compared with gastric tumors (20.9%) and in the intestinal subtype (32.4%) compared with a diffuse/mixed type (6.1%) [79].

Various case series suggest that HER2 overexpression or amplification is associated with worse prognosis [75, 80–84], whereas other studies have shown HER2 expression to have no prognostic significance at all [85, 86]. These conflicting results are likely related to historical challenges in measuring HER2 positivity in GC because these trials have used different scoring systems or methodologies to measure HER2 status. GC tumors have more heterogeneity in HER2 status compared with tumors of the breast, and HER2 staining in GC is more focal, with incomplete membrane staining [87]. Based on a study by Hofmann et al. [88] describing a scoring system for HER2 specific to GC, the ToGA trial modified how HER2 status was measured, and patients were eligible to be enrolled in the trial if the tumor had an IHC score of 3+ or positivity with fluorescence in situ hybridization (FISH; *HER2*-to-chromosome 17 ratio of $\geq$2.0). Recent guidelines recommend that when testing HER2 status in GC, IHC should be carried out first; 3+ is considered positive, and $\leq$1+ is negative. Samples scoring 2+ should move on to further testing with in situ hybridization, for which a *HER2*-to-chromosome 17 ratio of $\geq$2.0 is considered positive for gene amplification [87]. A recent study by Janjigian et al. attempted to clarify whether HER2 is a prognostic marker in Western patients with metastatic GC [89]. Using the guidelines outlined above, this study determined that HER2 status alone is not an independent prognostic marker.

HER2 has also been explored as a predictive biomarker in GC. Trastuzumab is a humanized murine monoclonal antibody that binds to the extracellular domain of the HER2 receptor. When trastuzumab binds to HER2, it blocks homo- or heterodimerization and causes receptor downregulation through increased endocytosis. It may also have an immune mechanism of action through the recruitment of antibodies, leading to cell-mediated toxicity [90].

The landmark ToGA study of patients with advanced HER2-positive GC was groundbreaking in the management of advanced GC because it substantiated the use of a targeted agent based on the measurement of a predictive biomarker. Patients were randomized to treatment with trastuzumab with cisplatin and a fluoropyrimidine or with cisplatin and a fluoropyrimidine alone [91]. Eighty percent of the 594 patients assigned to receive treatment had gastric tumors and 20% had GEJ tumors. The median OS in patients who received trastuzumab plus chemotherapy was 13.8 months compared with 11.1 months in the chemotherapy-alone group (HR: 0.74; 95% CI: 0.60–0.91; $p = .0046$). PFS was also significantly improved with the addition of trastuzumab to chemotherapy. A preplanned exploratory analysis suggested that patients with high expression of HER2 had a longer OS. In the 446 patients with IHC 3+ or IHC 2+ and FISH positivity, the median OS was 16 months among those who received trastuzumab and chemotherapy. The only increased grade 3 or 4 toxicity reported in the trastuzumab group was diarrhea. There was no difference in cardiac adverse events between the two arms, with $<$1% of patients experiencing cardiac failure in each study group. The median follow up was only 18.6 months, and patients will need to be followed for a longer duration of time to assess the long-term effects on cardiac function. The ToGA trial showed an improvement in OS with the use of trastuzumab plus chemotherapy compared with chemotherapy alone, and a subgroup analysis suggested that high HER2 protein expression is a predictive marker for survival benefit from this treatment. This is a new and exciting treatment based on a predictive marker with clear guidelines on how the marker should be measured; however, one may question how trastuzumab plus a fluoropyrimidine and cisplatin compares with three-drug chemotherapy regimens. As discussed above, a Cochrane meta-analysis showed a benefit with the addition of an anthracycline to CF [21], and the V325 study showed a clear survival benefit for DCF over CF [27]. It is recognized that three-drug chemotherapy regimens such as ECF, ECX, EOF, EOX, or DCF are difficult to administer because of toxicity and that patients must have minimal comorbidities and excellent PS to receive these treatments. In the ToGA trial, trastuzumab

with doublet chemotherapy was relatively well tolerated compared with chemotherapy alone, with the exception of more diarrhea in the trastuzumab group; however, >90% of enrolled patients had an ECOG score of 0–1. We will likely never know whether trastuzumab plus CF is better than trastuzumab plus triplet chemotherapy because of increased toxicity, particularly cardiotoxicity, as seen in the breast cancer population when trastuzumab was used concurrently with an anthracycline [92]. Given the cost of monoclonal antibody therapy, it is important to ask whether trastuzumab plus cisplatin and a fluorouracil is superior to a three-drug cytotoxic regimen. The ToGA trial has shown that HER2 positivity is indeed a predictive marker for response to trastuzumab, and the data suggest that when HER2 testing is available and done according to published guidelines for HER2 status in GC, trastuzumab plus a fluoropyrimidine and cisplatin should be offered to HER2-positive patients with good PS. In HER2-negative patients, depending on PS and comorbidities, triplet or doublet chemotherapy can be offered.

The ToGA trial has shown that HER2 positivity is indeed a predictive marker for response to trastuzumab, and the data suggest that when HER2 testing is available and done according to published guidelines for HER2 status in GC, trastuzumab plus a fluoropyrimidine and cisplatin should be offered to HER2-positive patients with good PS.

Lapatinib is an oral small molecule TKI of both EGFR and HER2. The multicenter phase III Lapatinib Optimization Study in ErbB2 (HER2) Positive Gastric Cancer (LOGiC) trial enrolled patients with advanced esophageal, GEJ, and gastric cancers with HER2 overexpression or amplification. Patients were randomized to first-line treatment with lapatinib plus chemotherapy with capecitabine and oxaliplatin versus chemotherapy alone, with a primary endpoint of OS. This study failed to demonstrate a statistically significant OS benefit to chemotherapy plus lapatinib over chemotherapy alone. Interestingly, a preplanned subgroup analysis showed a significant OS benefit in Asian patients and patients younger than 60 years old [93]. Further details, including clinical benefit and quality of life, are awaited in the publication of this trial.

Lapatinib plus paclitaxel versus paclitaxel alone was tested in the second-line setting for patients with GC with *HER2* amplification by FISH [94]. Although patients who received lapatinib along with chemotherapy had a 2-month improvement in OS, this difference was not statistically significant. Enrolled patients were required to have *HER2* amplification by FISH; however, 35% of patients had low IHC scores of 0 or 1+. When a preplanned subgroup analysis of patients with IHC 3+ tumors was done, there was a statistically significant benefit for OS (14 months for lapatinib plus paclitaxel versus 7.6 months for paclitaxel alone, $p = .0176$). The reason for the variability between FISH and IHC in this study is unclear. Based on the results of this study, lapatinib plus chemotherapy is not yet a convincing second-line option for patients with HER2-positive GC.

MET and Hepatocyte Growth Factor

MET is a tyrosine kinase receptor protein activated by the ligand hepatocyte growth factor (HGF) or scatter factor (SF) [95]. When HGF or SF activates MET, the receptor undergoes dimerization and phosphorylation, resulting in the activation of multiple pathways, including the phosphatidylinositol-3 kinase (PI3K)-protein kinase B (AKT) pathway and the RAS-mitogen activated protein kinase pathway. MET also has cross-talk with the EGFR and VEGFR pathways [96, 97]. Working together, these pathways signal cell survival, proliferation, migration [95], and metastasis [98]. *MET* amplification has also been implicated in TKI resistance in non-small cell lung cancer (NSCLC) [99]. It is known that MET kinase mutation or aberrant activation of MET is associated with renal cell carcinomas [100], and more recently, abnormalities in MET have been implicated in the pathogenesis of other solid tumors, including GC [101].

The MET protein is overexpressed in up to 50% of advanced GC tumors [82], and the *MET* gene is amplified in up to 20% of GCs [102, 103], although this number is lower, at <5% in Western populations [103, 104]. *MET* amplification or overexpression is a poor prognostic marker and is associated with more aggressive disease [82, 103, 105, 106].

MET overexpression has been demonstrated to be a predictive marker. Rilotumumab is a fully human monoclonal antibody targeting HGF or SF and preventing binding to the MET tyrosine kinase receptor. A randomized phase II clinical trial comparing rilotumumab at 15 mg/kg plus ECX chemotherapy versus rilotumumab at 7.5 mg/kg plus ECX versus ECX alone for the treatment of advanced gastric or GEJ cancer [107] showed that the addition of rilotumumab to ECX improved PFS. A biomarker analysis further demonstrated that MET overexpression is both a poor prognostic factor and a poor predictive factor. Patients with high tumor MET expression who received chemotherapy alone had worse OS [108]. Patients with high MET expression who received rilotumumab plus chemotherapy had improved OS compared with those who received chemotherapy alone (11.1 months versus 5.7 months). A phase III trial of rilotumumab in addition to chemotherapy is pending (ClinicalTrials.gov identifier NCT01697072).

Foretinib is a multikinase inhibitor of MET and VEGFRs that has been shown to be active in GC cell lines with MET activation [109]. In a phase II study of single-agent foretinib in unselected patients with advanced or metastatic GC, the best response was stable disease [110]. Approximately 20% of patients achieved stable disease for a median duration of 3.2 months. Only three of the patients in this study had tumor *MET* amplification. Further studies of foretinib in a selected population of patients with *MET* amplification are needed to assess whether targeting MET alone is a useful therapeutic strategy in GC or whether MET inhibitors should be combined with other targeted agents.

Crizotinib is another small molecule inhibitor of the MET kinase. Four patients with *MET*-amplified GC were enrolled in a phase I study of crizotinib [103]. Two of the 4 patients were enrolled from Asia and rapidly progressed before restaging. Of the other two patients, one was enrolled from the U.S. and one was enrolled from Australia. Both experienced a clinical response; one had a radiological partial response at 12 weeks, and the other patient exhibited stable disease.

MET is a promising target in GC. It is clear that MET overexpression is a poor prognostic factor, and early phase trials suggest that MET can be effectively targeted by agents such rilotumumab, foretinib, or crizotinib. Ongoing studies are currently investigating the role of targeting MET in the treatment of GC. Onartuzumab, a monoclonal antibody that binds to the MET receptor, is currently under investigation in the MetGastric trial [111], randomizing patients with MET overexpressing GC to 5-FU and oxaliplatin (the FOLFOX regimen) and onartuzumab versus FOLFOX alone, with a primary endpoint of OS. The MetGastric trial will accrue patients from North America, Latin America, Western Europe, Eastern Europe, and Asia, and results will be stratified by world region to address whether MET-targeted therapy differs between Eastern and Western populations. AMG 337 is an oral inhibitor of MET, and a phase II study (ClinicalTrials.gov identifier NCT02096666) is currently under way evaluating AMG 337 in patients with *MET*-amplified GC.

Mammalian Target of Rapamycin

Mammalian target of rapamycin (mTOR) is a protein kinase that controls the initiation of protein translation and thus is involved in regulating cell growth and survival [112–114]. mTOR activates S6 kinase 1 (S6K1), a kinase involved in protein synthesis, and inhibits the 4-E binding protein (4EBP1), a translational repressor [115] and possible tumor suppressor [116]. There is a suggestion that phosphorylated mTOR expression is a poor prognostic marker because it has been associated with worse disease-free survival in a small cohort [117]. Various cancers demonstrate abnormalities in mTOR or in its upstream regulators, such as PI3K, phosphatase and tensin homolog, and AKT; therefore, specific inhibitors of mTOR have been investigated as targeted cancer therapies [112, 114].

Everolimus is an oral inhibitor of mTOR. It prevents mTOR from phosphorylating S6K1 and 4EBP1, halting protein synthesis and blocking cell cycle progression [112, 115]. Higher expression of S6 proteins was associated with a higher disease control rate and longer PFS in a phase II second-line study of everolimus in GC [118]. The phase III Gastric Anti-Tumor Trial with Everolimus (GRANITE-1) study compared everolimus plus best supportive care with placebo plus best supportive care in patients with advanced GC who had progressed after one or two lines of chemotherapy [119]. Regardless of whether patients received everolimus or best supportive care, there was no significant difference in OS (5.4 months and 4.3 months, respectively) or time to deterioration in quality of life. Again, this trial included a heterogeneous group of patients. Patients from Asia and the rest of the world were included, and enrollment was not limited or stratified by predictive biomarkers. The heterogeneity of this group of patients may have masked any benefit with everolimus in the second- or third-line treatment of advanced GC. A biomarker analysis from the GRANITE-1 trial is pending and, hopefully, will shed light on whether any markers in the PI3K-AKT-mTOR pathway can be used to predict response to everolimus.

Potential New Targets

Many biomarkers that may have prognostic or predictive value have been evaluated in the preclinical setting. Protein p27 inhibits progression from gap 1 to the synthesis phase of the cell cycle. Retrospectively, GC tumors that are negative for p27 have been associated with higher rates of lymph node metastasis, a higher proliferative index, and worse OS, making p27 negativity a potential poor prognostic marker [120]. Metallothioneins are intracellular proteins involved in cell proliferation and apoptosis [121]. Low levels of metallothionein 2A have been associated with worse clinical outcomes [122]. CD44 and CD133 are both markers of cancer stem cells and are possible poor prognostic markers in GC [123–125]. Conversely, patients with tumors with microsatellite instability at multiple loci have a better prognosis [126]. Levels of apoptotic proteins may also be prognostic because patients with higher levels of the proapoptotic protein p53 and low levels of the antiapoptotic protein Bcl-2 may also be associated with improved survival [127, 128].

The significance of microRNA signatures as biomarkers is becoming apparent in many different malignancies. Various microRNAs have been useful for diagnostic purposes in GC, but less work has been done looking at the prognostic value. Elevated levels of miR-17-5p and miR-20 have been shown to correlate with poor OS and are potential poor prognostic markers [129]. Epigenetic alterations such as DNA methylation are also potential prognostic markers in GC that require further characterization and validation [130, 131].

Excision repair cross-complementation group 1 (ERCC1) is a protein involved in nucleotide excision repair (NER) of damaged DNA. Cisplatin binds to tumor DNA, creating platinum-DNA adducts, and ERCC1 is involved in recognizing and removing the platinum-DNA adducts as part of the NER process. ERCC1 positivity has been shown to be a poor predictive marker in NSCLC because patients with resected NSCLC who are ERCC1 positive do not derive any benefit from adjuvant platinum therapy, whereas low ERCC1 levels are associated with longer survival after adjuvant platinum chemotherapy [132]. Interestingly, ERCC1 is also a positive prognostic marker. In patients with surgically resected NSCLC who do not receive adjuvant chemotherapy, those with high ERCC1 expression experience longer survival than patients with low ERCC1 expression [133]

Preclinical data evaluating the predictive and prognostic value of ERCC1 in GC has revealed mixed results. Similar to in NSCLC, ERCC1 has been found to be a negative predictive factor in a European population receiving adjuvant cisplatin-based chemotherapy after gastrectomy because ERCC1 negativity was associated with longer OS [134]. Similar results were seen in an Asian population that received adjuvant oxaliplatin-based chemotherapy [135]. Again, similar to the data seen in NSCLC, elevated ERCC1 was a positive prognostic biomarker in patients who did not receive adjuvant chemotherapy after resection and a negative predictive marker in patients who received chemotherapy after gastrectomy [136]. The value of ERCC1 as a biomarker in the unresectable or metastatic GC setting is less clear. In a Korean population with advanced GC, ERCC1 negativity predicted increased response to oxaliplatin-based chemotherapy and longer OS [137]; however, ERCC1 status was not predictive in a Chinese population treated with DCF for advanced GC [138]. Low ERCC1 expression was associated with better PFS and OS in Japanese patients with advanced GC treated with chemotherapy [139]. This same study investigated whether ERCC1 could be used as a predictive

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marker for choosing between three different chemotherapy regimens: 5-FU alone, S1 alone, or irinotecan and cisplatin. There was a higher response rate but no difference in PFS in patients with low ERCC1 treated with irinotecan and cisplatin. ERCC1 levels did not help predict response to 5-FU or S1.

Another interesting predictive biomarker that has been investigated in GC is thymidylate synthase (TS). TS is the enzyme inhibited by 5-fluorouracil. Under normal conditions, TS converts deoxyuridine monophosphate to deoxythymidine monophosphate, resulting in the building blocks needed to form DNA. In a group of Japanese patients with metastatic GC treated with S1, low levels of TS predicted increased response to S1 and longer OS [140]. These results, however, were not confirmed in other studies of Asian patients with advanced GC treated with 5-FU-based chemotherapy [137, 141].

Adhesion molecule cadherin-17 is upregulated in GC and is a poor prognostic marker. Blocking cadherin-17 has been shown to inhibit tumor growth and invasion in mouse models; therefore, it is a potential predictive marker that warrants further therapeutic investigation [142].

Conclusion

Although there have been advances in the management of advanced and metastatic GC, the outcomes are still bleak. GC is a heterogeneous disease with different histologies and different anatomical presentations that vary around the world. Many potential prognostic and predictive biomarkers have been identified, and promising research is ongoing. Clinically relevant prognostic markers include VEGF, neuropilin, MET, and mTOR, in addition to many potential markers identified in the preclinical setting. The recognition of predictive biomarkers has been an exciting development in the management of GC. In particular, high expression of HER2 predicts for response to the humanized murine monoclonal antibody trastuzumab. The use of trastuzumab along with CF chemotherapy is the new standard of care for patients with HER2-positive GC. MET is another predictive marker because MET overexpression predicts response to rilotumumab and potentially to foretinib and crizotinib. Targeting angiogenesis is another exciting potential in GC. Although studies have not shown a role for the use of bevacizumab in GC, promising data suggest that VEGF or neuropilin may be useful as biomarkers to predict whether a selected group of patients may benefit from the use of bevacizumab. Ramucirumab, a monoclonal antibody against VEGFR-2, has now been shown to have a role in the second-line treatment of GC. The development of predictive biomarkers in GC is exciting because targeted therapy has the potential to completely change the landscape of how GC is managed. If biomarkers can be identified and verified in GC, this could lead to both better understanding of the pathogenesis and heterogeneity of GC and better outcomes for patients with this deadly disease.

Author Contributions

Conception/Design: Christina Kim, Karen Mulder, Jennifer Spratlin
Provision of study material or patients: Christina Kim
Collection and/or assembly of data: Christina Kim
Manuscript writing: Christina Kim
Final approval of manuscript: Christina Kim, Karen Mulder, Jennifer Spratlin

Disclosures

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